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(54) SYSTEMS AND METHODS FOR REAL TIME PREPARATION OF A POLYPEPTIDE SAMPLE FOR ANAYLSIS WITH MASS SPECTROMETRY

SYSTEME UND VERFAHREN ZUR ECHTZEIT PROBENVORBEREITUNG VON POLYPETIDES ZUR ANALYSE MITTELS MASSENSPEKTROMETRIE

SYSTÈMES ET PROCÉDÉS DE PRÉPARATION D'ÉCHANTILLON POLYPEPTIDES EN TEMPS RÉEL POUR ANAYLSE PAR SPECTROSCOPIE DE MASSE

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The complete document including Reference Table(s) and the Sequence Listing(s) can be downloaded from the EPO website

Description

FIELD OF THE DISCLOSURE

[0001] The present disclosure relates generally to assays and, more specifically, to performing a real-time assay of a sample.

SEQUENCE LISTING

[0002] The present application is being filed with a sequence listing in electronic format. The sequence listing provided as a file titled, "51800_Seqlisting.txt" created July 31, 2018 and is 263,964 bytes in size.

BACKGROUND

[0003] Assays are commonly performed to quantify one or more attributes of an analyte such as a drug, a biochemical substance, or a cell. An example of such an assay is the multi-attribute method (MAM) assay, which can detect and quantify Critical Quality Attributes (CQAs), identified by the Quality Target Product Profile (QTPP), of a sample (Development of a quantitative mass spectrometry multi-attribute method for characterization, quality control testing and disposition of biologics. Rogers RS, Nightlinger NS, Livingston B, Campbell P, Bailey R, Balland A. MAbs. 2015; 7(5): 881-90). The MAM assay is a manually-operated process that is performed in, for example, a Large Molecule Release Testing (LMRT) laboratory. MAM is a liquid chromatography (LC) - mass spectrometry (MS) -based peptide mapping method, comprising three steps: (1) sample preparation (such as polypeptide denaturation, reduction, alkylation, and digestion; (2) separation of the digested polypeptides by LC and detection by MS; and (3) analysis of the data for targeted CQAs and detection of new signal (*i.e.*, peaks) when compared to a reference standard. Hsieh Y L F et Al, Analytical Chemistry, American Chemical Society, vol. 68, no.3, pages 455-462, discloses an automated analytical system for the examination of protein primary structure.

[0004] CQAs are chemical, physical, or biological properties that are present within a specific value or range values. For example, for large polypeptide therapeutic molecules, physical attributes and modifications of amino acids (the building blocks of polypeptides) are important CQAs that are monitored during and after manufacturing, as well as during drug development. Unlike conventional analytical assays that track changes in peak size and peak shape of whole or partial polypeptides, MAM detects specific CQAs at the amino acid level.

[0005] However, while MAM is an important advance in the assessment of CQAs of polypeptide therapeutic molecules during development, manufacturing, and storage (assessing, *e.g.*, stability), analysis can consume seven to ten days, from sample preparation to final analysis, time that drives costs and delays development and drug release. For example, polypeptide therapeutics are customarily produced by cultured cells expressing the target polypeptide. Such production systems are not easily "put on hold" while MAM analysis of CQAs is pending, resulting in the cells producing the target polypeptide with CQAs that do not meet specifications - a waste of time, materials, and manpower. Furthermore, delays of seven to ten days accumulate during drug development, for example, when optimizing culture conditions, impeding delivery of important and new polypeptide pharmaceuticals to patients. Thus, there is a need for efficient and faster methods to facilitate CQA analysis using MAM.

SUMMARY

[0006] The invention is defined in the claims. One aspect of the present disclosure provides a method for performing a real-time assay. The method includes the steps of: (a) moving a sample of a product containing polypeptides to a polypeptide-binding column via a first holding coil; (b) binding the polypeptides in the sample to the polypeptide binding column, thereby separating the polypeptides in the sample from a remainder of the sample; (c) moving an elution buffer solution from a buffer source to the polypeptide binding column, via the first holding coil, and through a second holding coil downstream of the polypeptide binding column, thereby eluting polypeptides bound to the polypeptide binding column and moving an elution/polypeptide mixture comprising the elution buffer solution and the eluted polypeptides to the second holding coil; (d) moving the elution/polypeptide mixture from the second holding coil to a reaction chamber; (e) incubating the polypeptides in the elution/polypeptide mixture in the reaction chamber, resulting in denatured polypeptides; (f) moving a reducing reagent that cleaves disulfide bond crosslinks to the reaction chamber via the first holding coil after (e); (g) incubating the denatured polypeptides with the reducing reagent in the reaction chamber, resulting in denatured and reduced polypeptides; (h) moving an alkylating reagent that alkylates sulfhydryls to the reaction chamber after (g); (i) incubating the denatured and reduced polypeptides with the alkylating reagent in the reaction chamber, thereby alkylating the denatured and reduced polypeptides; (j) moving the denatured, reduced, and alkylated polypeptides, the elution buffer solution, the reducing reagent, and the alkylating reagent from the reaction chamber to

a desalting column via the first holding coil, the desalting column equilibrated with a proteolysis buffer; (k) applying the denatured, reduced, and alkylated polypeptides to the desalting column, thereby separating the denatured, reduced, and alkylated polypeptides from the reducing and alkylating reagents, and resulting in desalted polypeptides; (1) moving the desalted polypeptides to a proteolytic enzyme column downstream of the desalting column; (m) digesting the desalted polypeptides in the third polypeptide column, resulting in digested polypeptides; and (n) moving the digested polypeptides to an analytical device for analysis of the digested polypeptides.

[0007] Another aspect of the present disclosure provides a method for performing a real-time assay using a closed system including a multi-port valve, a first holding coil upstream of the multi-port valve, a polypeptide binding column fluidly coupled to and downstream of a first port of the multi-port valve, a second holding coil fluidly coupled to and downstream of the polypeptide binding column, a reaction chamber fluidly coupled to and downstream of second and third ports of the multi-port valve, a desalting column fluidly coupled to and downstream of a fourth port of the multi-port valve, and a proteolytic enzyme column downstream of the desalting column. The method includes: (a) moving, via a controller communicatively coupled to the closed system, a sample of a product containing polypeptides to the first holding coil; (b) positioning, via the controller, the multi-port valve in a first position in which the first holding coil is connected to the polypeptide binding column via the first port of the multi-port valve, such that the sample flows to the first column, whereby polypeptides in the sample bind to the polypeptide binding column; (c) when the multi-port valve is in the first position, moving, via the controller, an elution buffer solution from a source of elution buffer solution to the second holding coil via the polypeptide binding column, such that the elution buffer solution elutes substantially all of the polypeptides bound to the polypeptide binding column; (d) moving, via the controller, the multi-port valve to a second position in which the second holding coil is connected to the reaction chamber via the second port of the multi-port valve; (e) when the multi-port valve is in the second position, moving, via the controller, an elution/polypeptide mixture comprising the elution buffer solution and the eluted polypeptides to the reaction chamber, whereby the polypeptides in the elution/polypeptide mixture are denatured; (f) moving, via the controller, the multi-port valve to a third position in which the first holding coil is connected to the reaction chamber via the third port of the multi-port valve; (g) after (f), moving, via the controller, a reducing reagent that cleaves disulfide bond crosslinks to the first holding coil, and moving, via the controller, the reducing reagent from the first holding coil to the reaction chamber via the third port of the multi-port valve, thereby reducing the denatured polypeptides; (h) after (g), moving, via the controller, an alkylating reagent that alkylates sulfhydryl groups to the first holding coil, and moving, via the controller, the alkylating reagent from the first holding coil to the reaction chamber via the third port, thereby alkylating the denatured and reduced polypeptides; (i) moving, via the controller, the alkylated polypeptides, the elution buffer solution, the reducing reagent, and the alkylating reagent from the reaction chamber to the first holding coil via the third port; (j) moving, via the controller, the multi-port valve to a fourth position in which the first holding coil is connected to the desalting column via the fourth port of the multi-port valve, and, when the multi-port valve is in the fourth position, moving the alkylated polypeptides, the elution buffer solution, the reducing reagent, and the alkylating reagent from the first holding coil to the desalting column, whereby the denatured, reduced, and alkylated polypeptides are applied to the desalting column, thus separating the denatured, reduced, and alkylated polypeptides from the reducing and alkylating reagents, resulting in desalted polypeptides; (k) moving, via the controller, the desalted polypeptides to the proteolytic enzyme column, whereby the desalted polypeptides are digested; and (1) passing, via the controller, the digested polypeptides to an analytical device, whereby the digested polypeptides are analyzed.

[0008] Another aspect of the present disclosure provides a closed system for performing an online, real-time assay. The system includes: a first holding coil fluidly arranged to receive a sample of a product containing polypeptides; a multi-port valve fluidly coupled to and located downstream of the first holding coil; a polypeptide binding column fluidly coupled to the multi-port valve and arranged to receive the sample from the first holding coil via a first port of the multi-port valve, the polypeptide binding column configured to bind the polypeptides from the sample; a buffer source fluidly coupled to the multi-port valve and arranged to supply elution buffer solution to a second holding coil located downstream of the polypeptide binding column, such that the elution buffer solution is adapted to elute substantially all of the polypeptides from the polypeptide binding column; a reaction chamber fluidly coupled to the multi-port valve and arranged downstream of the polypeptide binding column, the reaction chamber adapted to receive a mixture from the polypeptide binding column via a second port of the multi-port valve, the mixture comprising the elution buffer solution and the eluted polypeptides, wherein the polypeptides of the mixture are denatured in the reaction chamber, wherein the reaction chamber is arranged to receive a reducing reagent that cleaves disulfide bond crosslinks via the first holding coil and a third port of the multi-port valve, the reducing reagent reduces the denatured polypeptides, and wherein the reaction chamber is further arranged to receive an alkylating reagent that alkylates sulfhydryls via the first holding coil and the third port of the multi-port valve, wherein the alkylating reagent alkylates the denatured and reduced polypeptides in the reaction chamber; a desalting column fluidly coupled to the multi-port valve and arranged to receive the denatured, reduced, and alkylated polypeptides, the elution buffer solution, and the alkylating reagent from the reaction chamber, the desalting column configured to separate the denatured, reduced, and alkylated polypeptides from the elution buffer solution, the reducing reagent, and the alkylating reagent; and a proteolytic enzyme column fluidly coupled to and

arranged downstream of the second polypeptide column to obtain the separated polypeptides from the desalting column, the proteolytic enzyme column configured to digest the desalted polypeptides.

[0009] Another aspect of the present disclosure provides a closed system for performing a real-time assay. The system includes: a multi-port valve; a first holding coil upstream of the multi-port valve; a polypeptide binding column fluidly coupled to and downstream of a first port of the multi-port valve; a second holding coil fluidly coupled to and downstream of the polypeptide binding column; a reaction chamber fluidly coupled to and downstream of second and third ports of the multi-port valve; a desalting column fluidly coupled to and downstream of a fourth port of the multi-port valve; a proteolytic enzyme column downstream of the desalting column; and a controller communicatively coupled to the multi-port valve. The controller includes a memory, a processor, and logic stored on the memory and executable by the processor to: (a) move a sample of a product containing polypeptides to the first holding coil; (b) position the multi-port valve in a first position in which the first holding coil is connected to the polypeptide binding column via the first port of the multi-port valve, such that the sample flows to the first column, whereby polypeptides in the sample bind to the polypeptide binding column; (c) when the multi-port valve is in the first position, move an elution buffer solution from a source of elution buffer solution to the second holding coil via the polypeptide binding column, such that the elution buffer solution elutes substantially all of the polypeptides bound to the polypeptide binding column; (d) move the multi-port valve to a second position in which the second holding coil is connected to the reaction chamber via the second port of the multi-port valve; (e) when the multi-port valve is in the second position, move an elution/polypeptide mixture comprising the elution buffer solution and the eluted polypeptides to the reaction chamber, whereby the polypeptides in the elution/polypeptide mixture are denatured; (f) move the multi-port valve to a third position in which the first holding coil is connected to the reaction chamber via the third port of the multi-port valve; (g) after (f), move a reducing reagent that cleaves disulfide bonds to the first holding coil, and move the reducing reagent from the first holding coil to the reaction chamber via the third port of the multi-port valve, thereby reducing the denatured polypeptides; (h) after (g), move an alkylating reagent that alkylates sulfhydryls to the first holding coil, and move the alkylating reagent from the first holding coil to the reaction chamber via the third port, thereby alkylating the denatured and reduced polypeptides; (i) move the denatured, reduced, and alkylated polypeptides, the elution buffer solution, the reducing reagent, and the alkylating reagent from the reaction chamber to the first holding coil via the third port; (j) move the multi-port valve to a fourth position in which the first holding coil is connected to the desalting column via the fourth port of the multi-port valve, and, when the multi-port valve is in the fourth position, move the denatured, reduced, and alkylated polypeptides, the elution buffer solution, the reducing reagent, and the alkylating reagent from the first holding coil to the desalting column, whereby the denatured, reduced, and alkylated polypeptides are de-salted; (k) move the desalted polypeptides to the proteolytic enzyme column, whereby the desalted polypeptides are digested; and (1) pass the digested polypeptides to a glycan analysis device, whereby the digested polypeptides are separated and quantified.

BRIEF DESCRIPTION OF THE DRAWINGS

[0010]

FIG. 1 is a schematic diagram of a system for performing an online, real-time assay assembled in accordance with the teachings of the present disclosure.

FIG. 2 is a schematic diagram of a controller of the system illustrated in FIG. 1.

FIGS. 3A and 3B are graphs depicting the results of a study monitoring the effectiveness of the system of FIG. 1 over a production run of 40 days.

FIG. 3C is a graph depicting a snapshot of the results of FIG. 3B over a 8 day period of time.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

[0011] The present disclosure provides for real-time assays and methods that allows for the monitoring and control of CQAs in real-time, so that the desired final polypeptide therapeutic product can be produced. The disclosed assays and methods can facilitate MAM result turn-around time such that results are available within a few hours (*e.g.*, two to three hours) instead of a typical, manually operated, off-line measurement result of seven to ten days. Thus, the disclosed assays and methods improve the turn-around time by approximately 54-fold to 120-fold. Such "on-the-fly" (real time) results enable, for example, adjusting production variables to manufacture efficiently (online) products having appropriate CQAs.

[0012] FIG. 1 illustrates a schematic diagram of a system 100 assembled in accordance with the teachings of the present disclosure. The system 100, which can be located at or in a laboratory (*e.g.,* a Large Molecule Release Testing laboratory) is a closed system for automatically or substantially automatically performing an assay of a sample of a product containing polypeptides, as is described in greater detail below. By automating (or substantially automating) this process using the system 100, the assay can be performed in real-time (or substantially in real-time, such that the

entire process can be performed, and the desired result obtained, in a matter of hours (*e.g.,* 2 to 3 hours), a significant improvement over the 7 to 10 days typically required by the conventionally known, manually-operated MAM assays. Moreover, the closed nature of the process that utilizes the system 100 maintains sterile conditions.

[0013] In this version, the polypeptides can be therapeutic polypeptides, which are discussed further below

[0014] The system 100 illustrated in FIG. 1 generally includes a multi-port valve 104, a first holding coil 108, a first column 112, a second holding coil 116, a reaction chamber 120, a second column 124, a third column 128, and a controller 132. In the version illustrated in FIG. 1, the system 100 also includes a vessel 136, a pump 140, a first waste chamber 144, a second waste chamber 148, a third waste chamber 152, and an analytical device 156 for analyzing the polypeptides. In other versions, however, the system 100 can not include one or more of these components. As an example, the system 100 can not include the vessel 136 and/or the analytical device 156. In any event, generally conventional plumbing extends between each of the components of the system 100 so as to facilitate fluid communication between components of the system 100 when desired, as is described in greater detail below. The multi-port valve 104 is generally configured to control fluid communication between the various components of the system 100. In this version, the multi-port valve 104 is a twelve satellite port and a central shared port valve. In other words, the multi-port valve 104 has a central port 160 and twelve satellite ports 164-175 that are selectively fluidly coupled to the central port 160. The multi-port valve 104 is movable between twelve different positions that fluidly couple the central port 160 with the twelve different satellite ports 164-175, respectively (some of which are not utilized in the operation of the system 100 of FIG. 1). In other versions, the multi-port valve 104 can include more or less satellite ports, can be a different type of valve or can be replaced by one or more different valves (each having one or more ports). As an example, the multi-port valve 104 can be replaced by a plurality of single port valves separately connected to and controlled by the controller 132, with each of the single port valves effectively replacing one of the satellite ports 164-175.

[0015] The vessel 136 is generally configured to hold or store the product containing polypeptides that is to be assayed. The vessel 136 in this version takes the form of a bioreactor that holds or stores the product. In other versions, however, the vessel 136 can instead take the form of a cell culture vessel, such as a flask, a plate, *etc.*. The vessel 136 is fluidly coupled to the satellite port 164 of the valve 104 via a conduit 180 of the plumbing, such that the valve 104 can, when desired, obtain a sample of the product contained in the vessel 136 from the vessel 136.

[0016] The first holding coil 108 is located upstream of the valve 104 and is fluidly coupled to the central port 160 of the valve 104 via a conduit 184 of the plumbing. The first holding coil 108 is thus arranged to receive the sample of the product from the vessel 136, via the valve 104, when the valve 104 is in a first position in which the central port 160 is fluidly coupled to the satellite port 164.

[0017] The first column 112 is located downstream of the valve 104 and is fluidly coupled to the satellite port 165 of the valve 104 via a conduit 188 of the plumbing. The first column 112 is thus arranged to receive the sample from the first holding coil 108, via the valve 104, when the valve 104 is in a second position in which the central port 160 is fluidly coupled to the satellite port 165. When the first column 112 receives the sample, the first column 112 is configured to bind polypeptides from the sample as the sample flows therethrough. In this manner, the first column 112 separates the polypeptides in the sample from a remainder of the sample, which can be passed to the first waste chamber 144 via the second holding coil 116.

[0018] The first column 112, which can also be referred to herein as a polypeptide-binding column, is selected from the group consisting of a protein A column, a protein G column, a protein A/G column, a protein L column, an amino acid column, an avidin column, a streptavidin column, a carbohydrate bonding column, a carbohydrate column, a glutathione column, a heparin column, a hydrophobic interaction column, an immunoaffinity column, a nucleotide/coenzyme column, a specialty column, and an immobilized-metal affinity chromatography (IMAC) column. For example, in the case of polypeptides that are human IgGs of subclasses 1, 2, or 4, IgM, IgA, or IgE (and comprising a human Fc portion and/or a Fab region of the human VH3 family), protein A columns are useful. Protein G columns can be used to purify human IgGs of subclasses 1-4. Recombinant fusion protein A/G columns can also be used to purify all of these classes of human antibodies, as the fusion protein provides protein A and protein G binding sites. Thus, protein A/G fusion proteins can be used to purify human IgG, IgA, IgE, and IgM. Furthermore, protein L columns can be used to purify human IgG, IgM, IgA, IgE and IgD, provided the target antibodies have an appropriate kappa (κ) subtype light chain (*i.e.*, VκI, VκIII and VκIV subtypes); protein L columns can also be used to purify Fab and scFv fragments also having the appropriate κ chain subtype, as protein L binds the variable (V) chain of antibodies.

[0019] The pump 140 is located upstream of and fluidly coupled to the first holding coil 108 via a conduit 192 of the plumbing. The pump 140 in this version takes the form of a syringe pump that is fluidly coupled to both a first buffer source 196 and a second buffer source 200. In this version, the first buffer source 196 is a elution buffer source that can supply an elution buffer solution 204, *e.g.,* in the case of protein A-bound antibodies, an acidic buffer; or in the case of protein G-bound antibodies, very acidic (pH 3 or less) buffer; one of skill in the art is able to optimize and select appropriate elution buffers for bound antibodies, to the pump 140 (and, ultimately, the first holding coil 108), and the second buffer source 200 is a denaturing buffer (containing a denaturant that disrupts quaternary, tertiary, or secondary polypeptide structure) source that can supply a denaturing reagent 208 to the pump 140 (and, ultimately, the first holding coil 108).

In other versions, the pump 140 can be a different type of pump and/or different pumps 140 can be used for each of the buffer sources 196, 200.

[0020] In some versions, the denaturing reagent can be or include a denaturing detergent or a chaotrope. In those versions in which the denaturing reagent is or includes a denaturing detergent, the denaturing detergent is preferably selected from the group consisting of sodium dodecyl sulfate (SDS), sodium cholate, sodium deoxycholate, sodium glycocholate, sodium taurocholate, sodium taurodeoxycholate, N-lauroylsarcosine, lithium dodecyl sulfate, hexadecyltrimethyl ammonium bromide (CTAB) and trimethyl(tetradecyl) ammonium bromide (TTAB). More preferably, the denaturing detergent is SDS. In those versions in which the denaturing reagent is or includes a chaotrope, the chaotrope is preferably selected from the group consisting of urea, n-butanol, ethanol, guanidinium chloride, lithium perchlorate, lithium acetate, magnesium chloride, phenol, 2-propanol, and thiourea. Alternatively or additionally, the denaturing reagent can be or include a heated fluid that has a temperature suitable for reaching, if not maintaining, a pre-determined temperature (*e.g.*, about 22 °C to about 120 °C) in the reaction chamber 120 when the denaturing reagent is passed to the reaction chamber 120.

[0021] A valve 212 is located between the pump 140 and the first and second buffer sources 196, 200 to selectively fluidly couple the pump 140 to only one of the buffer sources 196, 200 at a time. More particularly, the valve 212 is movable between a first position, in which the pump 140 is fluidly coupled to the first buffer source 196 and the pump 140 is fluidly isolated from the second buffer source 200, and a second position, in which the pump 140 is fluidly coupled to the second buffer source 200 and the pump 140 is fluidly isolated from the first buffer source 196. In other words, the pump 140 is selectively fluidly coupled to the first buffer source 196 or the second buffer source 200 depending upon the position of the valve 212.

[0022] As noted above, the pump 140 in this version is a syringe pump. The syringe pump 140 is generally configured to obtain a buffer from one of the buffer sources 196, 200 and output that buffer to the first holding coil 108. When the valve 212 is in the first position, the pump 140 can obtain (*e.g.*, draw in) the elution buffer solution 204 from the first buffer source 196, and, when desired, can output (*e.g.*, eject) that elution buffer solution 204 to the first holding coil 108. Conversely, when the valve 212 is in the second position, the pump 140 can obtain (*e.g.*, draw in) the denaturing reagent 208 from the second buffer source 200, and, when desired, can output (*e.g.*, eject) that denaturing reagent 208 to the first holding coil 108.

[0023] The second holding coil 116 is located downstream of the valve 104, the first holding coil 108, and the first column 112. The second holding coil 116 is selectively fluidly coupled to the satellite port 166 of the valve 104 via conduits 216, 220 of the plumbing. The second holding coil 116 is also selectively fluidly coupled to the first column 112 via the conduit 220 and a conduit 224. A three-way valve 228 is arranged between the conduits 216, 220, 224 to facilitate the selective coupling in order to produce the desired fluid flow, as is described in greater detail below.

[0024] The second holding coil 116 is thus arranged to receive the elution buffer solution 204 from the first holding coil 108, via the valve 104 and through the first column 112, when the pump 140 outputs the elution buffer solution 204 in the manner described above, the valve 104 is in the second position in which the central port 160 is fluidly coupled to the satellite port 165. As the elution buffer solution 204 passes through the first column 112, the elution buffer solution 204 elutes substantially all of the polypeptides bound to the first column 112. The three-way valve 228 is operated to connect the conduits 220, 224, thereby fluidly coupling the first column 112 with the second holding coil 116, such that an elution/polypeptide mixture including the elution buffer solution 204 and the eluted polypeptides flows from the first column 112 to the second holding coil 116 via the conduits 220, 224.

[0025] The reaction chamber 120 is located downstream of the valve 104 and the first column 112, and is fluidly coupled to the satellite port 167 of the valve 104 via conduit 232 of the plumbing. In this version, the reaction chamber 120 is partially, if not completely, pre-filled with the denaturing reagent 208 (*i.e.,* filled prior to operation of the system 100) from the second buffer source 200 by using the pump 140 to output the denaturing reagent 208 to the first holding coil 108 and moving the multi-port valve 104 to a fourth position in which the central port 160 is fluidly coupled to the satellite port 167 of the valve 167, thereby facilitating movement of the denaturing reagent 208 from the first holding coil 108 to the reaction chamber 120. In other versions, however, the reaction chamber 120 can be partially or completely filled with the denaturing reagent 208 during operation of the system 100 (*e.g.*, after the second holding coil 116 receives the elution buffer solution 204), partially or completely filled with a denaturing reagent from another buffer source and/or in a different manner, or the reaction chamber 120 may not be filled at all, in which case heat can be applied to the reaction chamber 120 by a heating element 236 connected to the reaction chamber 120.

[0026] After the elution/polypeptide mixture reaches the second holding coil 116, the elution/polypeptide mixture is moved to the reaction chamber 120. In this version, the reaction chamber 120 indirectly receives the elution/polypeptide mixture from the second holding coil 116. More particularly, the three-way valve 228 is operated to connect the conduits 216, 224, the elution/polypeptide mixture is moved from the second holding coil 116 to the satellite port 166 of the multi-port valve 104 via the conduits 216, 224, the valve 104 is moved to a third position in which the central port 160 is fluidly coupled to the satellite port 166, such that the mixture moves to the first holding coil 108, and the valve 104 is moved to a fourth position in which the central port 160 is fluidly coupled to the satellite port 167 of the valve 104, such that the

mixture moves from the first holding coil 108 to the reaction chamber 120. In other versions, the reaction chamber 120 can directly receive the elution/polypeptide mixture from the second holding coil 116 or can indirectly receive the mixture from the second holding coil 116 utilizing one or more different components and/or in a different order.

[0027] In any case, once the elution/polypeptide mixture reaches the reaction chamber 120, the polypeptides in the mixture incubate in the reaction chamber 120, thereby denaturing the polypeptides in the reaction chamber 120. When, for example, the reaction chamber 120 is at least partially filled with the denaturing reagent, the polypeptides in the mixture will, upon reaching the reaction chamber 120 and reacting with the denaturing reagent, undergo denaturation. In some cases, the reaction chamber 120 can, at the same time or at all times, be heated by the heating element 236 connected to (*e.g.*, positioned immediately adjacent, surrounding) the reaction chamber 120 to help facilitate the denaturation process. In other words, the heating element 236 can apply heat, preferably heat having a temperature of about 22 °C to about 120 °C and, more preferably, heat having a temperature of about 40 °C, to the reaction chamber 120 to encourage denaturation. The heating element 236 can, for example, take the form of a heating block, a heating coil, an induction heater, a heat pump, a cartridge heater, an electrical resistance wire, a heated fluid, or other element suitable for heating one or more portions of the reaction chamber 120. In any event, by applying heat from the heating element 236 to the reaction chamber 120, the process of denaturation can be facilitated. In other cases, however, the reaction chamber 120 can not be filled with the denaturing reagent and the denaturation process can be facilitated only by applying heat from the heating element 236.

[0028] Following the denaturation of the polypeptides in the mixture, the reaction chamber 120 is configured to receive a reducing reagent that cleaves disulfide bond crosslinks, thereby reducing the denatured polypeptides. The reducing reagent can be selected from the group consisting of dithiothreitol (DTT), glutathione, β-mercaptoethanol (β-ME), and tris(2-carboxyethyl)phosphine (TCEP) and is generally supplied by cooling vessel 240, which can, for example, take the form of a chiller having a temperature of 4 °C. In this version, the cooling vessel 240 is located downstream of the valve 104 and has a first chamber 242 that contains the reducing reagent and is fluidly coupled to the satellite port 168 of the valve 104 via conduit 244 of the plumbing. The first chamber 242 supplies the reducing reagent to the first holding coil 108 when the valve 104 is in a fifth position in which the central port 160 is fluidly coupled to the satellite port 168 of the valve 104, and the reducing reagent is then moved from the first holding coil 108 to the reaction chamber 120 when the valve 104 is in (or returns to) the fourth position (in which the central port 160 is fluidly coupled to the satellite port 167). Thus, in this version, the reducing reagent is indirectly supplied to the reaction chamber 120 via the first holding coil 108. In other versions, however, the first chamber 242 can directly supply the reducing reagent to the reaction chamber 120 (*i.e.,* without moving the reducing reagent to the first holding coil 108).

[0029] After the denatured polypeptides are reduced, the reaction chamber 120 is configured to receive an alkylating agent that alkylates sulfhydryls in the reaction chamber 120, thereby alkylating the denatured and reduced polypeptides in the reaction chamber 120. The alkylating agent is preferably an alkylating reagent such as indole-3-acetic acid (IAA), though other alkylating agents can be used. In this version, the cooling vessel 240 has a second chamber 248 that contains the alkylating agent and is fluidly coupled to the satellite port 169 of the valve 104 via conduit 252 of the plumbing. The second chamber 248 supplies the alkylating agent to the first holding coil 108 when the valve 104 is in a sixth position in which the central port 160 is fluidly coupled to the satellite port 169 of the valve 104, and the alkylating agent is then moved from the first holding coil 108 to the reaction chamber 120 when the valve 104 is in (or returns) to the fourth position. Thus, in this version, the alkylating agent is indirectly supplied to the reaction chamber 120 via the first holding coil 108. In other versions, however, the second chamber 248 can directly supply the alkylating agent to the reaction chamber 120 (*i.e*., without moving the alkylating agent to the first holding coil 108) and/or the alkylating agent can be supplied from a different cooling vessel (*e.g.*, a cooling vessel separate from the cooling vessel 240).

[0030] In this version, the system 100 further includes a first normally open valve 256 that is fluidly coupled to and located downstream of the reaction chamber 120. The normally open valve 256 has an inlet port 260 that is fluidly connected to an outlet 264 of the reaction chamber 120, a first outlet port 268 that is fluidly connected to the second waste chamber 148, and a second outlet port 272 that is fluidly connected to atmosphere. The normally open valve 256 normally operates in an open, or first, position when the system 100 is in operation and, more particularly, the reaction chamber 120 is receiving and being filled with the elution/polypeptide mixture, the reducing reagent, the alkylating agent, and, in some cases, the denaturing reagent, the valve 256. In this open position, the inlet port 260 is fluidly coupled to the first outlet port 268 and the inlet port 260 is fluidly isolated from the second outlet port 272, such that when the reaction chamber 120 is filled beyond its fixed volume, any excess contents are directed to the second waste chamber 148. However, the normally open valve 256 is movable (*e.g.*, by applying a current to the valve 256) from the open position to a closed, or second, position when, for example, the reaction chamber 120 is no longer receiving the above-described contents and it is time to clean the reaction chamber 120. In this closed position, the inlet port 260 is fluidly coupled to the second outlet port 272 and the inlet port 260 is fluidly isolated from the first outlet port 268, such that the outlet 264 of the reaction chamber 120 is exposed to the atmosphere. In turn, air can flow into the reaction chamber 120, thereby facilitating the removal of contents from the reaction chamber 120.

[0031] The second column 124, also referred to herein as the desalting column 124, preferably takes the form of a

size exclusion chromatography column that is located downstream of the valve 104 and is fluidly coupled to the satellite port 170 of the valve 104 via a conduit 276 of the plumbing. The second column 124 is thus arranged to receive the denatured, reduced, and alkylated polypeptides, the elution buffer solution 204, the alkylating agent, the reducing reagent, and the denaturing reagent (when one is used) from the reaction chamber 120. In this version, the second column 124 indirectly receives these materials from the reaction chamber 120. More particularly, these materials are moved from the reaction chamber 120 to the first holding coil 108, via the valve 104, when the valve 104 is in (or moved to) the fourth position (in which the central port 160 is fluidly coupled to the satellite port 167), and the valve 104 is moved to a seventh position in which the central port 160 is fluidly coupled to the satellite port 170, such that the materials move from the first holding coil 108 to the second column 124. In other versions, the second column 124 can directly receive these materials from the reaction chamber 120 or can indirectly receive these materials utilizing one or more different components and/or in a different order.

[0032] In any event, when the second column 124 receives the denatured, reduced, and alkylated polypeptides, the elution buffer solution 204, the alkylating agent, the reducing reagent, and the denaturing reagent (when one is used), the second column 124 is configured to separate the denatured, reduced, and alkylated polypeptides from the elution buffer solution 204, the alkylating agent, the reducing reagent, and the denaturing reagent (when used), which are ultimately moved to the third waste chamber 152. Thus, the second column 124 can be referred to herein as the desalting column 124. At the same time, the second column 124 is configured to buffer exchange the polypeptides into a desired buffer condition that allow the third column 128 to perform the functionality described below.

[0033] In this version, the system 100 further includes a second normally open valve 280 that is fluidly coupled to and located between the second column 124 and the third column 128. The normally open valve 280 has an inlet port 284 that is fluidly connected to an outlet 288 of the second column 124, a first outlet port 292 that is fluidly connected to the third waste chamber 152, and a second outlet port 296 that is fluidly connected to the third column 128. The normally open valve 280 normally operates in an open, or first, position when the system 100 is in operation. In this open position, the inlet port 284 is fluidly coupled to the first outlet port 292 and the inlet port 284 is fluidly isolated from the second outlet port 296, such that the elution buffer solution 204, the alkylating agent, the reducing reagent, and the denaturing reagent are directed to the third waste chamber 152. However, the normally open valve 280 is movable (*e.g.*, by applying a current to the valve 280) from the open position to a closed, or second, position when it is desired to move the desalted polypeptides from the second column 124 to the third column 128, which is located downstream of the second column 124. In this closed position, the inlet port 284 is fluidly coupled to the second outlet port 296 and the inlet port 284 is fluidly isolated from the first outlet port 292, such that the second column 124 is fluidly coupled to the third column 128, such that the desalted can pass from the second column 124 to the third column 128. In this version, the third column 128 includes a proteolytic enzyme (*e.g.*, an endopeptidase selected from the group consisting of trypsin, chymotrypsin, elastase, thermolysin, pepsin, glutamyl endopeptidase, neprilysin, Lys-C protease, and *Staphylococcus aureus* V8 protease), such that the third column 128 can be referred to herein as a proteolytic enzyme column. In any case, the third column 128 digests the desalted polypeptides obtained from the second column 124.

[0034] After the polypeptides have been digested in the third column 128, the digested polypeptides can be moved to the analytical device 156, which can, for example, take the form of a liquid chromatography device, a high-performance liquid chromatography device, an ultra high-performance liquid chromatography device, a mass spectrometry device, a glycan analysis device, another analysis device, or a combination thereof. In this version, the analytical device 156 is located downstream of the third column 128 and is fluidly coupled to the third column 128 via a conduit 298 of the plumbing. Thus, in this version, the digested polypeptides can be automatically moved to the analytical device 156 for analysis (*e.g.*, for quantification and separation). In other versions, however, the analytical device 156 is not be part of the system 100 (*e.g.*, not fluidly coupled to the third column 128), in which case the digested polypeptides can be moved to the analytical device 156 in a different manner (*e.g.*, manually).

[0035] As briefly noted above, the system 100 also includes the controller 132, which in this version is communicatively coupled or connected to various components of the system 100 to monitor and facilitate or direct the above-described operation of the system 100 by transmitting signals (*e.g.*, control signals, data) to and receiving signals (*e.g.*, data) from the various components of the system 100. The controller 132 can be located immediately adjacent the other components of the system 100 (*e.g.*, in the same environment as the system 100) or can be remotely located from the other components of the system 100. As illustrated, the controller 132 is communicatively coupled or connected to the multi-port valve 104 via a communication network 300, , the pump 140 via a communication network 328, the analytical device 156 via a communication network 332, the heating element 236 via a communication network 340, the first normally open valve 256 via a communication network 344, and the second normally open valve 280 via a communication network 348. In other versions, the controller 132 can be communicatively coupled or connected to more or less components of the system 100, *e.g.,* the first holding coil 108, the first column 112, the second holding coil 116, the reaction chamber 120, the second column 124, the third column 128, the vessel 136, the three-way valve 228, and/or the cooling vessel 240.

[0036] As used herein, the phrases "communicatively coupled" and "connected" are defined to mean directly coupled or connected to or indirectly coupled or connected through one or more intermediate components. Such intermediate

components can include hardware and/or software-based components. It is appreciated that the networks 300-348 can be wireless networks, wired networks, or combinations of a wired and a wireless network (*e.g.*, a cellular telephone network and/or 802.1 1x compliant network), and can include a publicly accessible network, such as the Internet, a private network, or a combination thereof. The type and configuration of the networks 300-348 is implementation dependent, and any type of communications networks which facilitate the described communications between the controller 132 and the components of the system 100, available now or later developed, can be used.

[0037] As shown in FIG. 2, the controller 132 includes a processor 352, a memory 356, a communications interface 360, and computing logic 364. The processor 352 can be a general processor, a digital signal processor, an application-specific integrated circuit (ASIC), field programmable gate array, graphics processing unit, analog circuit, digital circuit, or any other known or later developed processor. The processor 352 operates pursuant to instructions in the memory 356. The memory 356 can be a volatile memory or a non-volatile memory. The memory 356 can include one or more of a read-only memory (ROM), random-access memory (RAM), a flash memory, an electronic erasable program read-only memory (EEPROM), or other type of memory. The memory 356 can include an optical, magnetic (hard drive), or any other form of data storage device.

[0038] The communications interface 360 is provided to enable or facilitate electronic communication between the controller 132 and the components of the refrigeration system 100 via the networks 300-348. The communications interface 360 can be or include, for example, one or more universal serial bus (USB) ports, one or more Ethernet ports, and/or one or more other ports or interfaces. The electronic communication can occur via any known communications protocol, including, by way of example, USB, RS-232, RS-485, WiFi, Bluetooth, and/or any other suitable communications protocol.

[0039] The logic 364 generally includes one or more control routines and/or one or more sub-routines embodied as computer-readable instructions stored on the memory 356. The control routines and/or sub-routines can perform PID (proportional-integral-derivative), fuzzy logic, nonlinear, or any other suitable type of control. The processor 352 generally executes the logic 364 to perform actions related to the operation of the system 100.

[0040] Generally speaking, the logic 364, when executed, causes the processor 352 to control components of the system 100, particularly the multi-port valve 104, the pump 140, the heat element 236, the first and second normally open valves 256, 280, and the analytical device 156, such that the system 100 operates in the desired manner discussed herein. More particularly, the logic 364 can, when executed, cause the processor 352 to (i) move the multi-port valve 104 to or between any of the positions described herein, thereby fluidly coupling various components of the system 100 as described above, (ii) control the pump 140 (*e.g.*, cause the pump 140 to obtain and output the elution buffer solution 204 or the denaturing reagent 208), (iii) control the heating element 236 (when employed in the system 100) to selectively apply heat to the reaction chamber 120 (and the contents thereof), (iv) control the first normally open valve 256, (v) control the second normally open valve 280, (vi) control the analytical device 156, and perform other desired functionality.

[0041] When, for example, it is desired to perform a real-time assay of a sample of a product containing polypeptides, the logic 364 is executable by the processor 352 to position the valve 104 in the first position described above, move the sample of the product from the vessel 136 to the first holding coil 108 via the conduit 180, the port 164, the port 160, position the valve 104 in the second position described above, and move the sample from the first holding coil 108 to and through the polypeptide-binding column 112 via the conduit 184, the ports 160, 164, and the conduit 188. In turn, substantially all of the polypeptides in the sample bind to the column 112, such that the polypeptides in the sample are separated from the remainder of the sample, which passes to the first waste chamber 144 via the conduits 220, 224 and the second holding coil 116.

[0042] The logic 364 is further executable by the processor 352 to cause the pump 140 to obtain the elution buffer solution 204 from the first buffer source 196 and output the elution buffer solution 204 to the first holding coil 108 via the conduit 192. In some cases, the pump 140 can need to be moved from the second position to the first position (to fluidly couple the pump 140 with the first buffer source 196), but in other cases, the pump 140 can already be in the first position. In any case, the logic 364 is executable by the processor 352 to move the elution buffer solution 204 from the first holding coil 108 to and through the first column 112 and to the second holding coil 116, via the conduit 184, the ports 160, 165, and the conduits 188, 220, and 224. In this manner, the elution buffer solution 204 elutes substantially all of the polypeptides bound to the first column 112, and an elution/polypeptide mixture including the elution buffer solution 204 and the eluted polypeptides flows from the first column 112 to the second holding coil 116.

[0043] The logic 364 is further executable by the processor 352 to move the valve 104 to the third position described above, move the elution/polypeptide mixture from the second holding coil 116 to the first holding coil 108, via the conduits 224, 216, the ports 166, 160, and the conduit 184, move the valve 104 to the fourth position described above, and move the elution/polypeptide mixture from the first holding coil 108 to the reaction chamber 120, via the conduit 184, the ports 160, 167, and the conduit 232. In turn, the polypeptides in the elution/polypeptide mixture are incubated in the reaction chamber 120 with the denaturing reagent 208 and/or in the presence of heat applied by the heating element 236, which thereby denatures the polypeptides.

[0044] The logic 364 is further executable by the processor 352 to move the valve 104 to the fifth position described

above, move the reducing reagent from the first chamber 242 of the cooling vessel 240 to the first holding coil 108 via the conduit 244, the ports 168, 160, and the conduit 184, move the valve 104 back to the fourth position, and move the reducing reagent from the first holding coil 108 to the reaction chamber 120, via the conduit 184, the ports 160, 167, and the conduit 232. Upon reaching the reaction chamber 120, the reducing reagent cleaves disulfide bond crosslinks, which thereby reduces the denatured polypeptides in the reaction chamber 120.

[0045] The logic 364 is further executable by the processor 352 to move the valve 104 to the sixth position described above, move the alkylating agent from the second chamber 248 of the cooling vessel 240 to the first holding coil 108 via the conduit 252, the ports 169, 160, and the conduit 184, move the valve 104 back to the fourth position, and move the alkylating agent from the first holding coil 108 to the reaction chamber 120, via the conduit 184, the ports 160, 167, and the conduit 232. Upon reaching the reaction chamber 120, the alkylating agent alkylates sulfhydryls in the reaction chamber 120, which thereby alkylates the denatured and reduced polypeptides in the reaction chamber 120.

[0046] The logic 364 is further executable by the processor 352 to move the valve 104 to the fourth position (if not already there), move the denatured, reduced, and alkylated polypeptides, the elution buffer solution 204, the alkylating agent, the reducing reagent, and the denaturing reagent (when used) from the reaction chamber 120 to the first holding coil 108, via the conduit 232, the ports 167, 160, and the conduit 184, move the valve 104 to the seventh position described above, and move the denatured, reduced, and alkylated polypeptides, the elution buffer solution 204, the alkylating agent, the reducing reagent, and the denaturing reagent (when used) from the first holding coil 108 to the desalting column 124 via the conduit 184, the ports 160, 170, and the conduit 276. In turn, the desalting column 124 separates the denatured, reduced, and alkylated polypeptides from the elution buffer solution 204, the alkylating agent, the reducing reagent, and the denaturing reagent, which are passed or move to the third waste chamber 152.

[0047] At some point after the denatured, reduced, and alkylated polypeptides, the elution buffer solution 204, the alkylating agent, the reducing reagent, and the denaturing reagent (when used) are moved from the reaction chamber 120 to the first holding coil 108, the logic 364 is further executable by the processor 352 to move the normally open valve 256 from its open, first position, wherein the outlet 264 of the chamber 120 is fluidly coupled to the second waste chamber 148 so as to direct contents that will not fit in the reaction chamber 120 (as a result of it being filled beyond its fixed volume) to the second waste chamber 148, to its closed, second position, wherein the outlet 264 is fluidly coupled to atmosphere, such that air can flow into the reaction chamber 120, thereby facilitating the removal of contents from the reaction chamber 120. The normally open valve 256 can return to the open, first position immediately after the reaction chamber 120 has been emptied or can return to the open, first position at a later point in time.

[0048] After the desalting column 124 separates the denatured, reduced, and alkylated polypeptides from the other materials, the logic 364 is further executable by the processor 352 to move the normally open valve 280 from its open, first position, wherein the inlet port of the valve 280 is fluidly coupled to the third waste chamber 152, to its closed, second position, wherein the inlet port of the valve 280 is fluidly coupled to the proteolytic enzyme column 128. In turn, the logic 364 is executable by the processor 352 to move the desalted (or separated) polypeptides from the desalting column 124 to the proteolytic enzyme column 128, which digests the desalted polypeptides.

[0049] After the polypeptides have been digested, the logic 364 is, at least in this version, further executable by the processor 352 to move the digested polypeptides from the proteolytic enzyme column 128 to the analytical device 156 for analysis of the polypeptides, and to cause the analytical device 156 to perform the desired analysis. As an example, the logic 364 can, when executed by the processor 352, cause the analytical device 156 to separate and quantify the polypeptides.

[0050] In other versions, the logic 364 can, when executed by the processor 352, cause additional, less, and/or different functionality to be performed. As an example, the logic 364, when executed by the processor 352, may not move the digested polypeptides from the column 128 to the analytical device 156 or cause the analytical device 156 to perform the desired analysis. Moreover, in other versions, the logic 364 can be executed by the processor 352 in a different order than described herein. Finally, it is appreciated that the logic 364 can be executed by the processor 352 any number of different times, as the system 100 can be used to perform real-time analyses of multiple samples (from the same product and/or from a different product).

[0051] FIGS. 3A-3C illustrate the results of an online and real-time MAM assay study designed to monitor the effectiveness of the system 100 in preparing a sample of a product containing a Bispecific T-cell Engager (BiTE®) molecule. In particular, the study monitored the effectiveness of the system 100 over a production run of 40 days. The study began monitoring and collecting CQA data, such as area percentage for 2 deamidation sites, DS 1 and DS2, and the frequency of a fragmentation, FF, illustrated in FIG. 3A, and MS peak height (expressed as a number of ion counts) for four reference peptides RP1, RP2, RP3, and RP4, illustrated in FIGS. 3B and 3C, on day 6 of the 40-day production run. As illustrated in FIG. 3A, the system 100 capably and effectively performed the intended functionality discussed herein over the entire duration of the 40-day production run, and, as illustrated in FIGS. 3B and 3C, the CQA data collected between day 6 and day 40 was substantially consistent, *i.e.*, there was no significant change in product quality over time, and the product quality actually increased after day 32, thereby demonstrating the robustness of the system 100 in automatically preparing the sample. Indeed, as illustrated in FIG. 3C, the CQA data collected for RP1, RP2, RP3, and RP4 during that time-

period was better than the CQA data obtained during a typical manual MAM assay.

Therapeutic polypeptides

[0052] Proteins, including those that bind to one or more of the following, can be useful in the disclosed devices and methods. These include CD proteins, including CD3, CD4, CD8, CD19, CD20, CD22, CD30, and CD34; including those that interfere with receptor binding. HER receptor family proteins, including HER2, HER3, HER4, and the EGF receptor. Cell adhesion molecules, for example, LFA-I, MoI, pl50, 95, VLA-4, ICAM-I, VCAM, and alpha v/beta 3 integrin. Growth factors, such as vascular endothelial growth factor ("VEGF"), growth hormone, thyroid stimulating hormone, follicle stimulating hormone, luteinizing hormone, growth hormone releasing factor, parathyroid hormone, Mullerian-inhibiting substance, human macrophage inflammatory protein (MIP-I -alpha), erythropoietin (EPO), nerve growth factor, such as NGF-beta, platelet-derived growth factor (PDGF), fibroblast growth factors, including, for instance, aFGF and bFGF, epidermal growth factor (EGF), transforming growth factors (TGF), including, among others, TGF-α and TGF-β, including TGF-β1, TGF-β2, TGF-β3, TGF-β4, or TGF-β 5, insulin-like growth factors-I and -II (IGF-I and IGF-II), des(1-3)-IGF-I (brain IGF-I), and osteoinductive factors. Insulins and insulin-related proteins, including insulin, insulin A-chain, insulin B-chain, proinsulin, and insulin-like growth factor binding proteins. Coagulation and coagulation-related proteins, such as, among others, factor VIII, tissue factor, von Willebrands factor, protein C, alpha-1-antitrypsin, plasminogen activators, such as urokinase and tissue plasminogen activator ("t-PA"), bombazine, thrombin, and thrombopoietin; (vii) other blood and serum proteins, including but not limited to albumin, IgE, and blood group antigens. Colony stimulating factors and receptors thereof, including the following, among others, M-CSF, GM-CSF, and G-CSF, and receptors thereof, such as CSF-1 receptor (c-fms). Receptors and receptor-associated proteins, including, for example, flk2/flt3 receptor, obesity (OB) receptor, LDL receptor, growth hormone receptors, thrombopoietin receptors ("TPO-R," "c-mpl"), glucagon receptors, interleukin receptors, interferon receptors, T-cell receptors, stem cell factor receptors, such as c-Kit, and other receptors. Receptor ligands, including, for example, OX40L, the ligand for the OX40 receptor. Neurotrophic factors, including bone-derived neurotrophic factor (BDNF) and neurotrophin-3, -4, -5, or -6 (NT-3, NT-4, NT-5, or NT-6). Relaxin A-chain, relaxin B-chain, and prorelaxin; interferons and interferon receptors, including for example, interferon-α, -β, and -γ, and their receptors. Interleukins and interleukin receptors, including IL-I to IL-33 and IL-I to IL-33 receptors, such as the IL-8 receptor, among others. Viral antigens, including an AIDS envelope viral antigen. Lipoproteins, calcitonin, glucagon, atrial natriuretic factor, lung surfactant, tumor necrosis factor-alpha and -beta, enkephalinase, RANTES (regulated on activation normally T-cell expressed and secreted), mouse gonadotropin-associated peptide, DNAse, inhibin, and activin. Integrin, protein A or D, rheumatoid factors, immunotoxins, bone morphogenetic protein (BMP), superoxide dismutase, surface membrane proteins, decay accelerating factor (DAF), AIDS envelope, transport proteins, homing receptors, addressins, regulatory proteins, immunoadhesins, antibodies. Myostatins, TALL proteins, including TALL-I, amyloid proteins, including but not limited to amyloid-beta proteins, thymic stromal lymphopoietins ("TSLP"), RANK ligand ("OPGL"), c-kit, TNF receptors, including TNF Receptor Type 1, TRAIL-R2, angiopoietins, and biologically active fragments or analogs or variants of any of the foregoing.

[0053] Exemplary polypeptides and antibodies include Activase® (Alteplase); alirocumab, Aranesp® (Darbepoetin-alfa), Epogen® (Epoetin alfa, or erythropoietin); Avonex® (Interferon β-Ia); Bexxar® (Tositumomab); Betaseron® (Interferon-β); bococizumab (anti-PCSK9 monoclonal antibody designated as L1L3, see US8080243); Campath® (Alemtuzumab); Dynepo® (Epoetin delta); Velcade® (bortezomib); MLN0002 (anti-$\alpha 4\beta 7$ mAb); MLN1202 (anti-CCR2 chemokine receptor mAb); Enbrel® (etanercept); Eprex® (Epoetin alfa); Erbitux® (Cetuximab); evolocumab; Genotropin® (Somatropin); Herceptin® (Trastuzumab); Humatrope® (somatropin [rDNA origin] for injection); Humira® (Adalimumab); Infergen® (Interferon Alfacon-1); Natrecor® (nesiritide); Kineret® (Anakinra), Leukine® (Sargamostim); LymphoCide® (Epratuzumab); BenlystaTM (Belimumab); Metalyse® (Tenecteplase); Mircera® (methoxy polyethylene glycol-epoetin beta); Mylotarg® (Gemtuzumab ozogamicin); Raptiva® (efalizumab); Cimzia® (certolizumab pegol); SolirisTM (Eculizumab); Pexelizumab (Anti-C5 Complement); MEDI-524 (Numax®); Lucentis® (Ranibizumab); Edrecolomab (,Panorex®); Trabio® (lerdelimumab); TheraCim hR3 (Nimotuzumab); Omnitarg (Pertuzumab, 2C4); Osidem® (IDM-I); OvaRex® (B43.13); Nuvion® (visilizumab); Cantuzumab mertansine (huC242-DMl); NeoRecormon® (Epoetin beta); Neumega® (Oprelvekin); Neulasta® (Pegylated filgastrim, pegylated G-CSF, pegylated hu-Met-G-CSF); Neupogen® (Filgrastim); Orthoclone OKT3® (Muromonab-CD3), Procrit® (Epoetin alfa); Remicade® (Infliximab), Reopro® (Abciximab), Actemra® (anti-IL6 Receptor mAb), Avastin® (Bevacizumab), HuMax-CD4 (zanolimumab), Rituxan® (Rituximab); Tarceva® (Erlotinib); Roferon-A®-(Interferon alfa-2a); Simulect® (Basiliximab); StelaraTM (Ustekinumab); Prexige® (lumiracoxib); Synagis® (Palivizumab); 146B7-CHO (anti-IL15 antibody, see US7153507), Tysabri® (Natalizumab); Valortim® (MDX-1303, anti-B. anthracis Protective Antigen mAb); ABthraxTM; Vectibix® (Panitumumab); Xolair® (Omalizumab), ETI211 (anti-MRSA mAb), IL-I Trap (the Fc portion of human IgG1 and the extracellular domains of both IL-I receptor components (the Type I receptor and receptor accessory protein)), VEGF Trap (Ig domains of VEGFRl fused to IgG1 Fc), Zenapax® (Daclizumab); Zenapax® (Daclizumab), Zevalin® (Ibritumomab tiuxetan), Zetia (ezetimibe), Atacicept (TACI-Ig), anti-$\alpha 4\beta 7$ mAb (vedolizumab); galiximab (anti-CD80 monoclonal antibody), anti-CD23 mAb (lumiliximab); BR2-Fc (huBR3 / huFc fusion

protein, soluble BAFF antagonist); SimponiTM (Golimumab); Mapatumumab (human anti-TRAIL Receptor-1 mAb); Ocrelizumab (anti-CD20 human mAb); HuMax-EGFR (zalutumumab); M200 (Volociximab, anti-$\alpha 5\beta 1$ integrin mAb); MDX-010 (Ipilimumab, anti-CTLA-4 mAb and VEGFR-I (IMC-18F1); anti-BR3 mAb; anti-C. difficile Toxin A and Toxin B C mAbs MDX-066 (CDA-I) and MDX-1388); anti-CD22 dsFv-PE38 conjugates (CAT-3888 and CAT-8015); anti-CD25 mAb (HuMax-TAC); anti-TSLP antibodies; anti-TSLP receptor antibody (US8101182); anti-TSLP antibody designated as A5 (US7982016); (anti-CD3 mAb (NI-0401); Adecatumumab (MT201, anti-EpCAM-CD326 mAb); MDX-060, SGN-30, SGN-35 (anti-CD30 mAbs); MDX-1333 (anti- IFNAR); HuMax CD38 (anti-CD38 mAb); anti-CD40L mAb; anti-Cripto mAb; anti-CTGF Idiopathic Pulmonary Fibrosis Phase I Fibrogen (FG-3019); anti-CTLA4 mAb; anti-eotaxinl mAb (CAT-213); anti-FGF8 mAb; anti-ganglioside GD2 mAb; anti-sclerostin antibodies (see, US8715663 or US7592429) anti-sclerostin antibody designated as Ab-5 (US8715663 or US7592429); anti-ganglioside GM2 mAb; anti-GDF-8 human mAb (MYO-029); anti-GM-CSF Receptor mAb (CAM-3001); anti-HepC mAb (HuMax HepC); MEDI-545, MDX-1103 (anti-IFNα mAb); anti-IGFIR mAb; anti-IGF-IR mAb (HuMax-Inflam); anti-IL12/IL23p40 mAb (Briakinumab); anti-IL-23p19 mAb (LY2525623); anti-IL13 mAb (CAT-354); anti-IL-17 mAb (AIN457); anti-IL2Ra mAb (HuMax-TAC); anti-IL5 Receptor mAb; anti-integrin receptors mAb (MDX-O18, CNTO 95); anti-IPIO Ulcerative Colitis mAb (MDX- 1100); anti-LLY antibody; BMS-66513; anti-Mannose Receptor/hCGβ mAb (MDX-1307); anti-mesothelin dsFv-PE38 conjugate (CAT-5001); anti-PDImAb (MDX-1 106 (ONO- 4538)); anti-PDGFRα antibody (IMC-3G3); anti-TGFβ mAb (GC-1008); anti-TRAIL Receptor-2 human mAb (HGS-ETR2); anti-TWEAK mAb; anti-VEGFR/Flt-1 mAb; anti- ZP3 mAb (HuMax-ZP3); and an amyloid-beta monoclonal antibody comprising sequences, SEQ ID NO:8 and SEQ ID NO:6 (US7906625).

[0054] Examples of antibodies suitable for the methods and pharmaceutical formulations include the antibodies shown in Table 1. Other examples of suitable antibodies include infliximab, bevacizumab, cetuximab, ranibizumab, palivizumab, abagovomab, abciximab, actoxumab, adalimumab, afelimomab, afutuzumab, alacizumab, alacizumab pegol, ald518, alemtuzumab, alirocumab, altumomab, amatuximab, anatumomab mafenatox, anrukinzumab, apolizumab, arcitumomab, aselizumab, altinumab, atlizumab, atorolimiumab, tocilizumab, bapineuzumab, basiliximab, bavituximab, bectumomab, belimumab, benralizumab, bertilimumab, besilesomab, bevacizumab, bezlotoxumab, biciromab, bivatuzumab, bivatuzumab mertansine, blinatumomab, blosozumab, brentuximab vedotin, briakinumab, brodalumab, canakinumab, cantuzumab mertansine, cantuzumab mertansine, caplacizumab, capromab pendetide, carlumab, catumaxomab, cc49, cedelizumab, certolizumab pegol, cetuximab, citatuzumab bogatox, cixutumumab, clazakizumab, clenoliximab, clivatuzumab tetraxetan, conatumumab, crenezumab, cr6261, dacetuzumab, daclizumab, dalotuzumab, daratumumab, demcizumab, denosumab, detumomab, dorlimomab aritox, drozitumab, duligotumab, dupilumab, ecromeximab, eculizumab, edobacomab, edrecolomab, efalizumab, efungumab, elotuzumab, elsilimomab, enavatuzumab, enlimomab pegol, enokizumab, enoticumab, ensituximab, epitumomab cituxetan, epratuzumab, erenumab, erlizumab, ertumaxomab, etaracizumab, etrolizumab, evolocumab, exbivirumab, fanolesomab, faralimomab, farletuzumab, fasinumab, fbta05, felvizumab, fezakinumab, ficlatuzumab, figitumumab, flanvotumab, fontolizumab, foralumab, foravirumab, fresolimumab, fulranumab, futuximab, galiximab, ganitumab, gantenerumab, gavilimomab, gemtuzumab ozogamicin, gevokizumab, girentuximab, glembatumumab vedotin, golimumab, gomiliximab, gs6624, ibalizumab, ibritumomab tiuxetan, icrucumab, igovomab, imciromab, imgatuzumab, inclacumab, indatuximab ravtansine, infliximab, intetumumab, inolimomab, inotuzumab ozogamicin, ipilimumab, iratumumab, itolizumab, ixekizumab, keliximab, labetuzumab, lebrikizumab, lemalesomab, lerdelimumab, lexatumumab, libivirumab, ligelizumab, lintuzumab, lirilumab, lorvotuzumab mertansine, lucatumumab, lumiliximab, mapatumumab, maslimomab, mavrilimumab, matuzumab, mepolizumab, metelimumab, milatuzumab, minretumomab, mitumomab, mogamulizumab, morolimumab, motavizumab, moxetumomab pasudotox, muromonab-cd3, nacolomab tafenatox, namilumab, naptumomab estafenatox, narnatumab, natalizumab, nebacumab, necitumumab, nerelimomab, nesvacumab, nimotuzumab, nivolumab, nofetumomab merpentan, ocaratuzumab, ocrelizumab, odulimomab, ofatumumab, olaratumab, olokizumab, omalizumab, onartuzumab, oportuzumab monatox, oregovomab, orticumab, otelixizumab, oxelumab, ozanezumab, ozoralizumab, pagibaximab, palivizumab, panitumumab, panobacumab, parsatuzumab, pascolizumab, pateclizumab, patritumab, pemtumomab, perakizumab, pertuzumab, pexelizumab, pidilizumab, pintumomab, placulumab, ponezumab, priliximab, pritumumab, PRO 140, quilizumab, racotumomab, radretumab, rafivirumab, ramucirumab, ranibizumab, raxibacumab, regavirumab, reslizumab, rilotumumab, rituximab, robatumumab, roledumab, romosozumab, rontalizumab, rovelizumab, ruplizumab, samalizumab, sarilumab, satumomab pendetide, secukinumab, sevirumab, sibrotuzumab, sifalimumab, siltuximab, simtuzumab, siplizumab, sirukumab, solanezumab, solitomab, sonepcizumab, sontuzumab, stamulumab, sulesomab, suvizumab, tabalumab, tacatuzumab tetraxetan, tadocizumab, talizumab, tanezumab, taplitumomab paptox, tefibazumab, telimomab aritox, tenatumomab, tefibazumab, teneliximab, teplizumab, teprotumumab, tezepelumab, TGN1412, tremelimumab, ticilimumab, tildrakizumab, tigatuzumab, TNX-650, tocilizumab, toralizumab, tositumomab, tralokinumab, trastuzumab, TRBS07, tregalizumab, tucotuzumab celmoleukin, tuvirumab, ublituximab, urelumab, urtoxazumab, ustekinumab, vapaliximab, vatelizumab, vedolizumab, veltuzumab, vepalimomab, vesencumab, visilizumab, volociximab, vorsetuzumab mafodotin, votumumab, zalutumumab, zanolimumab, zatuximab, ziralimumab, zolimomab aritox.

[0055] Antibodies also include adalimumab, bevacizumab, blinatumomab, cetuximab, conatumumab, denosumab, eculizumab, erenumab, evolocumab, infliximab, natalizumab, panitumumab, rilotumumab, rituximab, romosozumab,

tezepelumab, and trastuzumab, and antibodies selected from Table 1.

Table 1

Examples of therapeutic antibodies

Target (informal name)	Conc. (mg/ml)	Viscosity (cP)	HC Type (including allotypes)	LC Type	pI	LC SEQ ID NO	HC SEQ ID NO
anti-amyloid	142.2	5.0	IgG1 (f) (R;EM)	Kappa	9.0	1	2
GMCSF (247)	139.7	5.6	IgG2	Kappa	8.7	3	4
CGRPR	136.6	6.3	IgG2	Lambda	8.6	5	6
RANKL	152.7	6.6	IgG2	Kappa	8.6	7	8
Sclerostin (27H6)	145.0	6.7	IgG2	Kappa	6.6	9	10
IL-1R1	153.9	6.7	IgG2	Kappa	7.4	11	12
Myostatin	141.0	6.8	IgG1 (z) (K;EM)	Kappa	8.7	13	14
B7RP1	137.5	7.7	IgG2	Kappa	7.7	15	16
Amyloid	140.6	8.2	IgG1 (za) (K;DL)	Kappa	8.7	17	18
GMCSF (3.112)	156.0	8.2	IgG2	Kappa	8.8	19	20
CGRP (32H7)	159.5	8.3	IgG2	Kappa	8.7	21	22
CGRP (3B6.2)	161.1	8.4	IgG2	Lambda	8.6	23	24
PCSK9 (8A3.1)	150.0	9.1	IgG2	Kappa	6.7	25	26
PCSK9 (492)	150.0	9.2	IgG2	Kappa	6.9	27	28
CGRP	155.2	9.6	IgG2	Lambda	8.8	29	30
Hepcidin	147.1	9.9	IgG2	Lambda	7.3	31	32
TNFR p55)	157.0	10.0	IgG2	Kappa	8.2	33	34
OX40L	144.5	10.0	IgG2	Kappa	8.7	35	36
HGF	155.8	10.6	IgG2	Kappa	8.1	37	38
GMCSF	162.5	11.0	IgG2	Kappa	8.1	39	40
Glucagon R	146.0	12.1	IgG2	Kappa	8.4	41	42
GMCSF (4.381)	144.5	12.1	IgG2	Kappa	8.4	43	44
Sclerostin (13F3)	155.0	12.1	IgG2	Kappa	7.8	45	46
CD-22	143.7	12.2	IgG1 (f) (R;EM)	Kappa	8.8	47	48
INFgR	154.2	12.2	IgG1 (za) (K;DL)	Kappa	8.8	49	50
Ang2	151.5	12.4	IgG2	Kappa	7.4	51	52
TRAILR2	158.3	12.5	IgG1 (f) (R;EM)	Kappa	8.7	53	54
EGFR	141.7	14.0	IgG2	Kappa	6.8	55	56
IL-4R	145.8	15.2	IgG2	Kappa	8.6	57	58
IL-15	149.0	16.3	IgG1 (f) (R;EM)	Kappa	8.8	59	60
IGF1R	159.2	17.3	IgG1 (za) (K;DL)	Kappa	8.6	61	62
IL-17R	150.9	19.1	IgG2	Kappa	8.6	63	64
Dkkl (6.37.5)	159.4	19.6	IgG2	Kappa	8.2	65	66
Sclerostin	134.8	20.9	IgG2	Kappa	7.4	67	68
TSLP	134.2	21.4	IgG2	Lambda	7.2	69	70
Dkk1 (11H10)	145.3	22.5	IgG2	Kappa	8.2	71	72
PCSK9	145.2	22.8	IgG2	Lambda	8.1	73	74
GIPR (2G10.006)	150.0	23.0	IgG1 (z) (K;EM)	Kappa	8.1	75	76
Activin	133.9	29.4	IgG2	Lambda	7.0	77	78
Sclerostin (2B8)	150.0	30.0	IgG2	Lambda	6.7	79	80
Sclerostin	141.4	30.4	IgG2	Kappa	6.8	81	82
c-fms	146.9	32.1	IgG2	Kappa	6.6	83	84
$\alpha 4\beta 7$	154.9	32.7	IgG2	Kappa	6.5	85	86

* An exemplary concentration suitable for patient administration; ^HC - antibody heavy chain; LC - antibody light chain.

[0056] Based on the foregoing description, it should be appreciated that the devices, systems, and methods described herein facilitate the performance of an assay of a sample substantially in real-time. Thus, the assay can be performed, and the desired result obtained, much more quickly than allowed by conventional processes.

[0057] It should also be appreciated that the devices, systems, and methods described herein allow the process of preparing the online, real-time assay using the system 100 to be easily monitored, which can in turn mitigate risk and extend a production run of the product. In particular, this process can be monitored by determining, e.g., using a controller such as the controller 132 and/or manually by an operator of the system 100, whether conditions in the system 100 are optimal, i.e., whether they satisfy a pre-determined performance threshold. As illustrated in FIGS. 3B and 3C, for example, MS Peak Height, expressed in ion counts, for the four reference peptides RP1, RP2, RP3, and RP4 can be obtained and compared against historical values for those reference peptides during the method qualification to determine whether conditions in the system 100 are optimal (and they are), such that the production run can be commenced, continued, or extended. However, when it is determined that the conditions in the system 100 are not optimal, at least one cell culture component can be adjusted until the conditions in the closed system are optimal. Examples of cell culture components that can be adjusted include, but are not limited to: pH, pressure, temperature, media flow (*e.g.*, media flow rate, media feed rate), media content (including amino acids, nutrients, sugars, buffer), gassing strategy (*e.g.,* mix of oxygen and carbon dioxide, gas rate), agitation (*e.g.*, agitation rate), additives (e.g., metal additives, sugar additives), additive (*e.g.*, anti-foam) feed rate, and perfusion rate. In some cases, only one cell culture component may need to be adjusted so that the conditions in the closed system are optimal, while in other cases, multiple cell culture components may need to be adjusted. Alternatively, when it is determined that the conditions are not optimal, or when the conditions in the system 100 are not optimal even after adjusting the at least one cell culture component, the controller and/or the operator of the system 100 may shut down the system 100.

[0058] In this manner, the devices, systems, and methods described herein also mitigate risk involved in the continued operation of the system 100 when conditions are not optimal or when it is otherwise undesirable to continue operation of the system 100. In particular, risk can be mitigated by determining (*e.g.*, calculating or obtaining) process parameter and product quality data, *e.g.*, pH, temperature, oxygen dissolution, cell viability, cell density, titer, aggregation, charge variant, glycosylation, etc., associated with the current operation of the system 100, determining whether the process parameter and product quality data satisfy a pre-determined risk threshold (determined before operation of the system 100 by a controller such as the controller 132 and/or responsive to input from the operator of the system 100), and then determining whether to continue, cease, or adjust operation of the system 100 based upon whether the process parameter and product quality data satisfy the pre-determined risk threshold. The pre-determined risk threshold may, for example, be determined by looking at (1) applying available process parameter data and product quality data to calculate average historical multi-variate (MV) data associated with the previous operation of the system 100 or some other similar system, and then (2) establishing the calculated average historical MV data as the threshold (the threshold may be calculated average historical MV data itself or some value or set of values based on the calculated average historical MV data. In one example, the pre-determined risk threshold may represent an acceptable deviation (e.g., three standard deviations) from the calculated average historical MV data. Alternatively or additionally, the pre-determined risk threshold may be determined based upon input from the operator of the system 100. In some cases, the system 100 may be shut down when the process parameter and product quality data associated with the current operation of the system 100 do not satisfy (*e.g.*, exceed) the pre-determined risk threshold. In other cases, however, the system 100 may be adjusted when the process parameter and product quality data associated with the current operation of the system 100 do not satisfy (*e.g.*, exceed) the pre-determined risk threshold or even when the process parameter and product quality data satisfy but are close to the pre-determined risk threshold.

[0059] Further, the Applicant has discovered that the devices, systems, and methods described herein also allow production runs using the system 100 to be extended. Conventional processes typically allow for 32 to 40-day production runs, at most. However, the Applicant has found that the devices, systems, and methods described herein allow for 50-80 if not 100 population doublings, *i.e.*, approximately 50-80 if not 100 day production runs. Thus, more product can be obtained, all while operation of the system 100 is monitored to ensure that the product satisfies quality objectives and risk is mitigated.

[0060] Preferred embodiments of this disclosure are described herein, including the best mode or modes known to the inventors for carrying out the disclosure. Although numerous examples are shown and described herein, those of skill in the art will readily understand that details of the various embodiments need not be mutually exclusive. Instead, those of skill in the art upon reading the teachings herein should be able to combine one or more features of one embodiment with one or more features of the remaining embodiments. Further, it also should be understood that the illustrated embodiments are exemplary only, and should not be taken as limiting the scope of the disclosure. All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of any and all examples, or exemplary language (*e.g.*, "such as") provided herein, is intended merely to better illuminate the aspects of the exemplary embodiment or embodiments of the disclosure, and do not pose a limitation on the scope of the disclosure. No language in the specification should be construed as indicating any non-

claimed element as essential to the practice of the disclosure.

Claims

1. A method for performing a real-time assay, the method comprising the steps of:

   (a) moving a sample of a product containing polypeptides to a polypeptide-binding column (112) via a first holding coil (108);
   (b) binding the polypeptides in the sample to the polypeptide binding column, thereby separating the polypeptides in the sample from a remainder of the sample;
   (c) moving an elution buffer solution from a buffer source to the polypeptide binding column, via the first holding coil, and through a second holding coil (116) downstream of the polypeptide binding column, thereby eluting polypeptides bound to the polypeptide binding column and moving an elution/polypeptide mixture comprising the elution buffer solution and the eluted polypeptides to the second holding coil;
   (d) moving the elution/polypeptide mixture from the second holding coil to a reaction chamber (120);
   (e) incubating the polypeptides in the elution/polypeptide mixture in the reaction chamber, resulting in denatured polypeptides;
   (f) moving a reducing reagent that cleaves disulfide bond crosslinks to the reaction chamber via the first holding coil after (e);
   (g) incubating the denatured polypeptides with the reducing reagent in the reaction chamber, resulting in denatured and reduced polypeptides;
   (h) moving an alkylating reagent that alkylates sulfhydryls to the reaction chamber after (g);
   (i) incubating the denatured and reduced polypeptides with the alkylating reagent in the reaction chamber, thereby alkylating the denatured and reduced polypeptides;
   (j) moving the denatured, reduced, and alkylated polypeptides, the elution buffer solution, the reducing reagent, and the alkylating reagent from the reaction chamber to a desalting column (124) via the first holding coil, the desalting column equilibrated with a proteolysis buffer;
   (k) applying the denatured, reduced, and alkylated polypeptides to the desalting column, thereby separating the denatured, reduced, and alkylated polypeptides from the reducing and alkylating reagents, and resulting in desalted polypeptides;
   (l) moving the desalted polypeptides to a proteolytic enzyme column (128) downstream of the desalting column;
   (m) digesting the desalted polypeptides in the proteolytic enzyme column, resulting in digested polypeptides; and
   (n) moving the digested polypeptides to an analytical device (156) for analysis of the digested polypeptides.

2. The method of claim 1, wherein one or more of (a), (c), (d), (f), (h), (i), (l), and (n) are performed automatically; and/or wherein (a) through (n) are performed in a closed system.

3. The method of claims 1 or 2, wherein (a) comprises positioning a multi-port valve (104) in a first position in which the first holding coil and the polypeptide binding column are fluidly coupled via a first port of the multi-port valve; optionally wherein (d) comprises positioning the multi-port valve in a second position in which the second holding coil and the reaction chamber are fluidly coupled via a second port of the multi-port valve.

4. The method of claim 3, wherein (f) and (h) each comprises positioning the multi-port valve in a third position in which the first holding coil and the reaction chamber are fluidly coupled via a third port of the multi-port valve; optionally wherein (j) comprises:

   moving the denatured, reduced, and alkylated polypeptides, the elution buffer solution, the reducing reagent, and the alkylating reagent from the reaction chamber to the desalting column via the third port of the multi-port valve; and
   positioning the multi-port valve in a fourth position in which the first holding coil and the desalting column are fluidly coupled via a fourth port of the multi-port valve.

5. The method of any one of claims 1 to 4, further comprising:

   prior to or during (d), moving a first valve (256) fluidly coupled to and located downstream of the reaction chamber to a first position in which the first valve directs contents received from the first holding coil in excess of a volume of the reaction chamber to a waste chamber, optionally wherein the method further comprises, after (i), moving

the first valve from the first position to a second position in which the first valve directs air into the reaction chamber; and/or

after (k) and before (l), (o) moving a second valve (280) fluidly coupled to and located between the desalting and proteolytic enzyme columns from a first position, in which the second valve directs contents received from the desalting column to a waste chamber, to a second position, in which the second valve directs contents received from the desalting column to the proteolytic enzyme column; and/or

wherein (a) comprises moving the sample of the product from a vessel containing the polypeptides to the polypeptide-binding column via the first holding coil; and/or

prior to (d), at least partially filling the reaction chamber with a denaturing reagent, and wherein (e) comprises incubating the polypeptides in the elution/polypeptide mixture with the denaturing reagent in the reaction chamber, optionally wherein at least partially filling the reaction chamber with the denaturing reagent comprises moving the denaturing reagent from a denaturing buffer source to the reaction chamber via the first holding coil, or optionally wherein (j) further comprises moving the denaturing reagent from the reaction chamber to the desalting column via the first holding coil, and wherein (k) further separates the denatured, reduced, and alkylated polypeptides from the denaturing reagent; and/or

applying heat to the reaction chamber.

6. A method for performing a real-time assay using a closed system comprising a multi-port valve (104), a first holding coil (108) upstream of the multi-port valve, a polypeptide binding column (112) fluidly coupled to and downstream of a first port of the multi-port valve, a second holding coil (116) fluidly coupled to and downstream of the polypeptide binding column, a reaction chamber (120) fluidly coupled to and downstream of second and third ports of the multi-port valve, a desalting column fluidly coupled to and downstream of a fourth port of the multi-port valve, and a proteolytic enzyme column (128) downstream of the desalting column, the method comprising:

(a) moving, via a controller communicatively coupled to the closed system, a sample of a product containing polypeptides to the first holding coil;

(b) positioning, via the controller, the multi-port valve in a first position in which the first holding coil is connected to the polypeptide binding column via the first port of the multi-port valve, such that the sample flows to the first column, whereby polypeptides in the sample bind to the polypeptide binding column;

(c) when the multi-port valve is in the first position, moving, via the controller, an elution buffer solution from a source of elution buffer solution to the second holding coil via the polypeptide binding column, such that the elution buffer solution elutes substantially all of the polypeptides bound to the polypeptide binding column;

(d) moving, via the controller, the multi-port valve to a second position in which the second holding coil is connected to the reaction chamber via the second port of the multi-port valve;

(e) when the multi-port valve is in the second position, moving, via the controller, an elution/polypeptide mixture comprising the elution buffer solution and the eluted polypeptides to the reaction chamber, whereby the polypeptides in the elution/polypeptide mixture are denatured;

(f) moving, via the controller, the multi-port valve to a third position in which the first holding coil is connected to the reaction chamber via the third port of the multi-port valve;

(g) after (f), moving, via the controller, a reducing reagent that cleaves disulfide bond crosslinks to the first holding coil, and moving, via the controller, the reducing reagent from the first holding coil to the reaction chamber via the third port of the multi-port valve, thereby reducing the denatured polypeptides;

(h) after (g), moving, via the controller, an alkylating reagent that alkylates sulfhydryl groups to the first holding coil, and moving, via the controller, the alkylating reagent from the first holding coil to the reaction chamber via the third port, thereby alkylating the denatured and reduced polypeptides;

(i) moving, via the controller, the alkylated polypeptides, the elution buffer solution, the reducing reagent, and the alkylating reagent from the reaction chamber to the first holding coil via the third port;

(j) moving, via the controller, the multi-port valve to a fourth position in which the first holding coil is connected to the desalting column via the fourth port of the multi-port valve, and, when the multi-port valve is in the fourth position, moving the alkylated polypeptides, the elution buffer solution, the reducing reagent, and the alkylating reagent from the first holding coil to the desalting column, whereby the denatured, reduced, and alkylated polypeptides are applied to the desalting column, thus separating the denatured, reduced, and alkylated polypeptides from the reducing and alkylating reagents, resulting in desalted polypeptides;

(k) moving, via the controller, the desalted polypeptides to the proteolytic enzyme column, whereby the desalted polypeptides are digested; and

(l) passing, via the controller, the digested polypeptides to an analytical device (156), whereby the digested polypeptides are analyzed.

7. The method of claim 6, wherein (a) comprises moving, via the controller, the sample from a vessel containing the polypeptides to the first holding coil; and/or
wherein (k) comprises passing the digested polypeptides to an analytical device selected from the group consisting of a liquid chromatography device, a high-performance liquid chromatography device, an ultra high-performance liquid chromatography device, a mass spectrometry device, and a glycan analysis device, or a combination thereof.

8. The method of claim 6 or 7, further comprising, prior to (e), moving, via the controller, the multi-port valve to the third position, and moving, via the controller, a denaturing reagent to the reaction chamber.

9. The method of claim 8, wherein moving the denaturing reagent to the reaction chamber comprises: moving, via the controller, the denaturing reagent from a denaturing buffer source to the first holding coil via a pump, and moving, via the controller, the denaturing reagent from the first holding coil to the reaction chamber via the third port of the multi-port valve.

10. The method of claim 8 or 9, wherein (i) further comprises moving the denaturing reagent from the reaction chamber to the first holding coil via the third port, and wherein (j) further comprises moving the denaturing reagent from the first holding coil to the desalting column, and whereby when the denatured, reduced, and alkylated polypeptides are applied to the desalting column, the denatured, reduced, and alkylated polypeptides are further separated from the denaturing reagent.

11. A closed system for performing an online, real-time assay, the system comprising:

a first holding coil fluidly (108) arranged to receive a sample of a product containing polypeptides;
a multi-port valve (104) fluidly coupled to and located downstream of the first holding coil;
a polypeptide binding column (112) fluidly coupled to the multi-port valve and arranged to receive the sample from the first holding coil via a first port of the multi-port valve, the polypeptide binding column configured to bind the polypeptides from the sample,
a buffer source fluidly coupled to the multi-port valve and arranged to supply elution buffer solution to a second holding coil located downstream of the polypeptide binding column, such that the elution buffer solution is adapted to elute substantially all of the polypeptides from the polypeptide binding column;
a reaction chamber (120) fluidly coupled to the multi-port valve and arranged downstream of the polypeptide binding column, the reaction chamber adapted to receive a mixture from the polypeptide binding column via a second port of the multi-port valve, the mixture comprising the elution buffer solution and the eluted polypeptides, wherein the polypeptides of the mixture are denatured in the reaction chamber, wherein the reaction chamber is arranged to receive a reducing reagent that cleaves disulfide bond crosslinks via the first holding coil and a third port of the multi-port valve, the reducing reagent reduces the denatured polypeptides, and wherein the reaction chamber is further arranged to receive an alkylating reagent that alkylates sulfhydryls via the first holding coil and the third port of the multi-port valve, wherein the alkylating reagent alkylates the denatured and reduced polypeptides in the reaction chamber;
a desalting column (124) fluidly coupled to the multi-port valve and arranged to receive the denatured, reduced, and alkylated polypeptides, the elution buffer solution, and the alkylating reagent from the reaction chamber, the desalting column configured to separate the denatured, reduced, and alkylated polypeptides from the elution buffer solution, the reducing reagent, and the alkylating reagent; and
a proteolytic enzyme column (128) fluidly coupled to and arranged downstream of the second polypeptide column to obtain the separated polypeptides from the desalting column, the proteolytic enzyme column configured to digest the desalted polypeptides.

12. A closed system for performing a real-time assay, the system comprising:

a multi-port valve (104);
a first holding coil (108) upstream of the multi-port valve;
a polypeptide binding column (112) fluidly coupled to and downstream of a first port of the multi-port valve;
a second holding coil (116) fluidly coupled to and downstream of the polypeptide binding column;
a reaction chamber (120) fluidly coupled to and downstream of second and third ports of the multi-port valve;
a desalting column (124) fluidly coupled to and downstream of a fourth port of the multi-port valve;
a proteolytic enzyme column (128) downstream of the desalting column; and
a controller (132) communicatively coupled to the multi-port valve and comprising a memory, a processor, and logic stored on the memory and executable by the processor to:

(a) move a sample of a product containing polypeptides to the first holding coil;
(b) position the multi-port valve in a first position in which the first holding coil is connected to the polypeptide binding column via the first port of the multi-port valve, such that the sample flows to the first column, whereby polypeptides in the sample bind to the polypeptide binding column;
(c) when the multi-port valve is in the first position, move an elution buffer solution from a source of elution buffer solution to the second holding coil via the polypeptide binding column, such that the elution buffer solution elutes substantially all of the polypeptides bound to the polypeptide binding column;
(d) move the multi-port valve to a second position in which the second holding coil is connected to the reaction chamber via the second port of the multi-port valve;
(e) when the multi-port valve is in the second position, move an elution/polypeptide mixture comprising the elution buffer solution and the eluted polypeptides to the reaction chamber, whereby the polypeptides in the elution/polypeptide mixture are denatured;
(f) move the multi-port valve to a third position in which the first holding coil is connected to the reaction chamber via the third port of the multi-port valve;
(g) after (f), move a reducing reagent that cleaves disulfide bonds to the first holding coil, and move the reducing reagent from the first holding coil to the reaction chamber via the third port of the multi-port valve, thereby reducing the denatured polypeptides;
(h) after (g), move an alkylating reagent that alkylates sulfhydryls to the first holding coil, and move the alkylating reagent from the first holding coil to the reaction chamber via the third port, thereby alkylating the denatured and reduced polypeptides;
(i) move the denatured, reduced, and alkylated polypeptides, the elution buffer solution, the reducing reagent, and the alkylating reagent from the reaction chamber to the first holding coil via the third port;
(j) move the multi-port valve to a fourth position in which the first holding coil is connected to the desalting column via the fourth port of the multi-port valve, and, when the multi-port valve is in the fourth position, move the denatured, reduced, and alkylated polypeptides, the elution buffer solution, the reducing reagent, and the alkylating reagent from the first holding coil to the desalting column, whereby the denatured, reduced, and alkylated polypeptides are de-salted;
(k) move the desalted polypeptides to the proteolytic enzyme column, whereby the desalted polypeptides are digested; and
(l) pass the digested polypeptides to a glycan analysis device, whereby the digested polypeptides are separated and quantified.

13. The method of any one of claims 1-10 or the system of claims 11-12, wherein the polypeptide of the product is a therapeutic polypeptide;
optionally wherein the therapeutic polypeptide is selected from the group consisting of an antibody or antigen-binding fragment thereof, a derivative of an antibody or antibody fragment, and a fusion polypeptide.

14. The method or system of claim 13, wherein the antibody is selected from the group consisting of infliximab, bevacizumab, ranibizumab, cetuximab, ranibizumab, palivizumab, abagovomab, abciximab, actoxumab, adalimumab, afelimomab, afutuzumab, alacizumab, alacizumab pegol, ald518, alemtuzumab, alirocumab, alemtuzumab, altumomab, amatuximab, anatumomab mafenatox, anrukinzumab, apolizumab, arcitumomab, aselizumab, altinumab, atlizumab, atorolimiumab, tocilizumab, bapineuzumab, basiliximab, bavituximab, bectumomab, belimumab, benralizumab, bertilimumab, besilesomab, bevacizumab, bezlotoxumab, biciromab, bivatuzumab, bivatuzumab mertansine, blinatumomab, blosozumab, brentuximab vedotin, briakinumab, brodalumab, canakinumab, cantuzumab mertansine, cantuzumab mertansine, caplacizumab, capromab pendetide, carlumab, catumaxomab, cc49, cedelizumab, certolizumab pegol, cetuximab, citatuzumab bogatox, cixutumumab, clazakizumab, clenoliximab, clivatuzumab tetraxetan, conatumumab, crenezumab, cr6261, dacetuzumab, daclizumab, dalotuzumab, daratumumab, demcizumab, denosumab, detumomab, dorlimomab aritox, drozitumab, duligotumab, dupilumab, ecromeximab, eculizumab, edobacomab, edrecolomab, efalizumab, efungumab, elotuzumab, elsilimomab, enavatuzumab, enlimomab pegol, enokizumab, enokizumab, enoticumab, enoticumab, ensituximab, epitumomab cituxetan, epratuzumab, erlizumab, ertumaxomab, etaracizumab, etrolizumab, exbivirumab, exbivirumab, fanolesomab, faralimomab, farletuzumab, fasinumab, fbta05, felvizumab, fezakinumab, ficlatuzumab, figitumumab, flanvotumab, fontolizumab, foralumab, foravirumab, fresolimumab, fulranumab, futuximab, galiximab, ganitumab, gantenerumab, gavilimomab, gemtuzumab ozogamicin, gevokizumab, girentuximab, glembatumumab vedotin, golimumab, gomiliximab, gs6624, ibalizumab, ibritumomab tiuxetan, icrucumab, igovomab, imciromab, imgatuzumab, inclacumab, indatuximab ravtansine, infliximab, intetumumab, inolimomab, inotuzumab ozogamicin, ipilimumab, iratumumab, itolizumab, ixekizumab, keliximab, labetuzumab, lebrikizumab, lemalesomab, lerdelimumab, lexatumumab, libivirumab, ligelizumab, lintuzumab, lirilumab, lorvotuzumab mertansine, lucatumumab, lumiliximab, mapatumumab, maslimomab, mavrili-

mumab, matuzumab, mepolizumab, metelimumab, milatuzumab, minretumomab, mitumomab, mogamulizumab, morolimumab, motavizumab, moxetumomab pasudotox, muromonab-cd3, nacolomab tafenatox, namilumab, naptumomab estafenatox, narnatumab, natalizumab, nebacumab, necitumumab, nerelimomab, nesvacumab, nimotuzumab, nivolumab, nofetumomab merpentan, ocaratuzumab, ocrelizumab, odulimomab, ofatumumab, olaratumab, olokizumab, omalizumab, onartuzumab, oportuzumab monatox, oregovomab, orticumab, otelixizumab, oxelumab, ozanezumab, ozoralizumab, pagibaximab, palivizumab, panitumumab, panobacumab, parsatuzumab, pascolizumab, pateclizumab, patritumab, pemtumomab, perakizumab, pertuzumab, pexelizumab, pidilizumab, pintumomab, placulumab, ponezumab, priliximab, pritumumab, PRO 140, quilizumab, racotumomab, radretumab, rafivirumab, ramucirumab, ranibizumab, raxibacumab, regavirumab, reslizumab, rilotumumab, rituximab, robatumumab, roledumab, romosozumab, rontalizumab, rovelizumab, ruplizumab, samalizumab, sarilumab, satumomab pendetide, secukinumab, sevirumab, sibrotuzumab, sifalimumab, siltuximab, simtuzumab, siplizumab, sirukumab, solanezumab, solitomab, sonepcizumab, sontuzumab, stamulumab, sulesomab, suvizumab, tabalumab, tacatuzumab tetraxetan, tadocizumab, talizumab, tanezumab, taplitumomab paptox, tefibazumab, telimomab aritox, tenatumomab, tefibazumab, telimomab aritox, tenatumomab, teneliximab, teplizumab, teprotumumab, tezepelumab, TGN1412, tremelimumab, ticilimumab, tildrakizumab, tigatuzumab, TNX-650, tocilizumab, toralizumab, tositumomab, tralokinumab, trastuzumab, TRBS07, tregalizumab, tremelimumab, tucotuzumab celmoleukin, tuvirumab, ublituximab, urelumab, urtoxazumab, ustekinumab, vapaliximab, vatelizumab, vedolizumab, veltuzumab, vepalimomab, vesencumab, visilizumab, volociximab, vorsetuzumab mafodotin, votumumab, zalutumumab, zanolimumab, zatuximab, ziralimumab, zolimomab aritox, and those antibodies shown in Table 1.

15. The method or system of claim 13, wherein the therapeutic polypeptide is a polypeptide selected from the group consisting of a glycoprotein, CD polypeptide, a HER receptor polypeptide, a cell adhesion polypeptide, a growth factor polypeptide, an insulin polypeptide, an insulin-related polypeptide, a coagulation polypeptide, a coagulation-related polypeptide, albumin, IgE, a blood group antigen, a colony stimulating factor, a receptor, a neurotrophic factor, an interferon, an interleukin, a viral antigen, a lipoprotein, calcitonin, glucagon, atrial natriuretic factor, lung surfactant, tumor necrosis factor-alpha and -beta, enkephalinase, mouse gonadotropin-associated peptide, DNAse, inhibin, activing, an integrin, protein A, protein D, a rheumatoid factor, an immunotoxin, a bone morphogenetic protein, a superoxide dismutase, a surface membrane polypeptide, a decay accelerating factor, an AIDS envelope, a transport polypeptide, a homing receptor, an addressin, a regulatory polypeptide, an immunoadhesin, a myostatin, a TALL polypeptide, an amyloid polypeptide, a thymic stromal lymphopoietin, a RANK ligand, a c-kit polypeptide, a TNF receptor, and an angiopoietin, and biologically active fragments, analogs or variants thereof.

Patentansprüche

1. Verfahren zum Durchführen eines Echtzeit-Assays, wobei das Verfahren die Schritte umfasst:

(a) Bewegen einer Probe eines polypeptidenthaltenden Produkts zu einer polypeptidbindenden Säule (112) über eine erste Haltespule (108);
(b) Binden der Polypeptide in der Probe an die polypeptidbindende Säule, wobei dadurch die Polypeptide in der Probe von einem Rest der Probe getrennt werden;
(c) Bewegen einer Elutionspufferlösung von einer Pufferquelle zu der polypeptidbindenden Säule über die erste Haltespule und durch eine zweite Haltespule (116) stromabwärts der polypeptidbindenden Säule, wobei dadurch an die polypeptidbindende Säule gebundene Polypeptide eluiert werden und eine Elutions-/Polypeptidmischung, die die Elutionspufferlösung und die eluierten Polypeptide umfasst, zu der zweiten Haltespule bewegt wird;
(d) Bewegen der Elutions-/Polypeptidmischung von der zweiten Haltespule zu einer Reaktionskammer (120);
(e) Inkubieren der Polypeptide in der Elutions-/Polypeptidmischung in der Reaktionskammer, was zu denaturierten Polypeptiden führt;
(f) Bewegen eines reduzierenden Reagens, das Disulfidbindungsvernetzungen spaltet, zu der Reaktionskammer über die erste Haltespule nach (e);
(g) Inkubieren der denaturierten Polypeptide mit dem reduzierenden Reagens in der Reaktionskammer, was zu denaturierten und reduzierten Polypeptiden führt;
(h) Bewegen eines Alkylierungsreagens, das Sulfhydrylate alkyliert, zu der Reaktionskammer nach (g);
(i) Inkubieren der denaturierten und reduzierten Polypeptide mit dem Alkylierungsreagens in der Reaktionskammer, wobei dadurch die denaturierten und reduzierten Polypeptide alkyliert werden;
(j) Bewegen der denaturierten, reduzierten und alkylierten Polypeptide, der Elutionspufferlösung, des reduzierenden Reagens und des alkylierenden Reagens von der Reaktionskammer zu einer Entsalzungssäule (124) über die erste Haltespule, wobei die Entsalzungssäule mit einem Proteolysepuffer äquilibriert ist;

(k) Aufbringen der denaturierten, reduzierten und alkylierten Polypeptide auf die Entsalzungssäule, wobei dadurch die denaturierten, reduzierten und alkylierten Polypeptide von dem reduzierenden und Alkylierungsreagens getrennt werden und entsalzte Polypeptide erhalten werden;
(l) Bewegen der entsalzten Polypeptide zu einer proteolytischen Enzymsäule (128) stromabwärts der Entsalzungssäule;
(m) Verdauen der entsalzten Polypeptide in der proteolytischen Enzymsäule, was zu verdauten Polypeptiden führt; und
(n) Bewegen der verdauten Polypeptide zu einer Analysevorrichtung (156) für die Analyse der verdauten Polypeptide.

2. Verfahren nach Anspruch 1, wobei einer oder mehrere von (a), (c), (d), (f), (h), (i), (j) und (n) automatisch durchgeführt werden; und/oder
wobei (a) bis (n) in einem geschlossenen System durchgeführt werden.

3. Verfahren nach Anspruch 1 oder 2, wobei (a) das Positionieren eines Mehrwegeventils (104) in einer ersten Position umfasst, in der die erste Haltespule und die polypeptidbindende Säule über einen ersten Anschluss des Mehrwegeventils fluidisch gekoppelt sind;
optional wobei (d) das Positionieren des Mehrwegeventils in einer zweiten Position umfasst, in der die zweite Haltespule und die Reaktionskammer über einen zweiten Anschluss des Mehrwegeventils fluidisch gekoppelt sind.

4. Verfahren nach Anspruch 3, wobei (f) und (h) jeweils das Positionieren des Mehrwegeventils in einer dritten Position umfassen, in der die erste Haltespule und die Reaktionskammer über einen dritten Anschluss des Mehrwegeventils fluidisch gekoppelt sind;
optional wobei (j) umfasst:

Bewegen der denaturierten, reduzierten und alkylierten Polypeptide, der Elutionspufferlösung, des reduzierenden Reagens und des Alkylierungsreagens von der Reaktionskammer zu der Entsalzungssäule über den dritten Anschluss des Mehrwegeventils; und
Positionieren des Mehrwegeventils in einer vierten Position, in der die erste Haltespule und die Entsalzungssäule über einen vierten Anschluss des Mehrwegeventils fluidisch gekoppelt sind.

5. Verfahren nach einem der Ansprüche 1 bis 4, das ferner umfasst:

vor oder während (d), Bewegen eines ersten Ventils (256), das mit der Reaktionskammer fluidisch gekoppelt und sich stromabwärts von dieser befindet, in eine erste Position, in der das erste Ventil von der ersten Haltespule aufgenommenen Inhalt, der das Volumen der Reaktionskammer übersteigt, in eine Abfallkammer leitet, optional wobei das Verfahren ferner nach (i) das Bewegen des ersten Ventils von der ersten Position in eine zweite Position umfasst, in der das erste Ventil Luft in die Reaktionskammer leitet; und/oder
nach (k) und vor (l), (o) Bewegen eines zweiten Ventils (280), das mit der Entsalzungs- und der proteolytischen Enzymsäule fluidisch gekoppelt ist und sich zwischen diesen befindet, von einer ersten Position, in der das zweite Ventil den von der Entsalzungssäule aufgenommenen Inhalt zu einer Abfallkammer leitet, in eine zweite Position, in der das zweite Ventil den von der Entsalzungssäule aufgenommenen Inhalt zu der proteolytischen Enzymsäule leitet; und/oder
wobei (a) das Bewegen der Produktprobe von einem die Polypeptide enthaltenden Gefäß zu der polypeptidbindenden Säule über die erste Haltespule umfasst; und/oder
vor (d) das wenigstens teilweise Füllen der Reaktionskammer mit einem Denaturierungsreagens, und wobei (e) das Inkubieren der Polypeptide in der Elutions/Polypeptidmischung mit dem Denaturierungsreagens in der Reaktionskammer umfasst, wobei optional das wenigstens teilweise Füllen der Reaktionskammer mit dem Denaturierungsreagens das Bewegen des Denaturierungsreagens von einer Denaturierungspufferquelle zu der Reaktionskammer über die erste Haltespule umfasst, oder optional, wobei (j) ferner das Bewegen des Denaturierungsreagens von der Reaktionskammer zu der Entsalzungssäule über die erste Haltespule umfasst, und wobei (k) ferner die denaturierten, reduzierten und alkylierten Polypeptide von dem Denaturierungsreagens trennt; und/oder
Anwenden von Wärme auf die Reaktionskammer.

6. Verfahren zum Durchführen eines Echtzeit-Assays unter Verwendung eines geschlossenen Systems, das ein Mehrwegeventil (104), eine erste Haltespule (108) stromaufwärts des Mehrwegeventils, eine polypeptidbindende Säule (112), die mit einem ersten Anschluss des Mehrwegeventils fluidisch gekoppelt ist und stromabwärts von diesem

angeordnet ist, eine zweite Haltespule (116), die mit der polypeptidbindende Säule fluidisch gekoppelt ist und stromaufwärts von dieser angeordnet ist, eine Reaktionskammer (120), die mit einem zweiten und einem dritten Anschluss des Mehrwegeventils fluidisch gekoppelt ist und stromabwärts von diesen angeordnet ist, eine Entsalzungssäule, die mit einem vierten Anschluss des Mehrwegeventils fluidisch gekoppelt ist und stromabwärts von diesem angeordnet ist, und eine proteolytische Enzymsäule (128) stromabwärts von der Entsalzungssäule, wobei das Verfahren umfasst:

(a) Bewegen einer Probe eines Produkts, das Polypeptide enthält, über eine Steuervorrichtung, die kommunikativ mit dem geschlossenen System verbunden ist, zu der ersten Haltespule;
(b) Positionieren des Mehrwegeventils über die Steuervorrichtung in einer ersten Position, in der die erste Haltespule über den ersten Anschluss des Mehrwegeventils mit der polypeptidbindenden Säule verbunden ist, sodass die Probe zu der ersten Säule fließt, wodurch Polypeptide in der Probe an die polypeptidbindende Säule binden;
(c) wenn sich das Mehrwegeventil in der ersten Position befindet, Bewegen einer Elutionspufferlösung von einer Quelle für Elutionspufferlösung über die polypeptidbindende Säule zu der zweiten Haltespule über die Steuervorrichtung, sodass die Elutionspufferlösung im Wesentlichen alle an die polypeptidbindende Säule gebundenen Polypeptide eluiert;
(d) Bewegen des Mehrwegeventils über die Steuervorrichtung in eine zweite Position, in der die zweite Haltespule über den zweiten Anschluss des Mehrwegeventils mit der Reaktionskammer verbunden ist;
(e) wenn sich das Mehrwegeventil in der zweiten Position befindet, Bewegen einer Elutions/Polypeptidmischung, die die Elutionspufferlösung und die eluierten Polypeptide umfasst, über die Steuervorrichtung in die Reaktionskammer, wodurch die Polypeptide in der Elutions/Polypeptidmischung denaturiert werden;
(f) Bewegen des Mehrwegeventils über die Steuervorrichtung in eine dritte Position, in der die erste Haltespule über den dritten Anschluss des Mehrwegeventils mit der Reaktionskammer verbunden ist;
(g) nach (f), Bewegen eines reduzierenden Reagens, das Disulfidbindungsvernetzungen spaltet, über die Steuervorrichtung zu der ersten Haltespule und Bewegen des reduzierenden Reagens über die Steuervorrichtung von der ersten Haltespule zu der Reaktionskammer über den dritten Anschluss des Mehranschlussventils, wobei dadurch die denaturierten Polypeptide reduziert werden;
(h) nach (g), Bewegen eines Alkylierungsreagens, das Sulfhydrylgruppen alkyliert, über die Steuervorrichtung zu der ersten Haltespule und Bewegen des Alkylierungsreagens von der ersten Haltespule über die dritte Öffnung zu der Reaktionskammer über die Steuervorrichtung, wobei dadurch die denaturierten und reduzierten Polypeptide alkyliert werden;
(i) Bewegen der alkylierten Polypeptide, der Elutionspufferlösung, des reduzierenden Reagens und des Alkylierungsreagens über die Steuervorrichtung von der Reaktionskammer zu der ersten Haltespule über den dritten Anschluss;
(j) Bewegen des Mehrwegeventils über die Steuervorrichtung in eine vierte Position, in der die erste Haltespule über den vierten Anschluss des Mehrwegeventils mit der Entsalzungssäule verbunden ist, und, wenn sich das Mehrwegeventil in der vierten Position befindet, Bewegen der alkylierten Polypeptide, der Elutionspufferlösung, des Reduktionsreagens, und des Alkylierungsreagens von der ersten Haltespule zu der Entsalzungssäule, wodurch die denaturierten, reduzierten und alkylierten Polypeptide auf die Entsalzungssäule aufgebracht werden, wodurch die denaturierten, reduzierten und alkylierten Polypeptide von den reduzierenden und alkylierenden Reagenzien getrennt werden, was zu entsalzten Polypeptiden führt;
(k) Bewegen der entsalzten Polypeptide über die Steuervorrichtung zu der proteolytischen Enzymsäule, wodurch die entsalzten Polypeptide verdaut werden; und
(l) Passieren der verdauten Polypeptide über die Steuervorrichtung an eine Analysevorrichtung (156), wodurch die verdauten Polypeptide analysiert werden.

7. Verfahren nach Anspruch 6, wobei (a) das Bewegen der Probe aus einem Gefäß, das die Polypeptide enthält, über die Steuervorrichtung zu der ersten Haltespule umfasst; und/oder
wobei (k) das Passieren der verdauten Polypeptide zu einer Analysevorrichtung umfasst, die aus der Gruppe ausgewählt ist, die aus einer Flüssigkeitschromatographievorrichtung, einer Hochleistungsflüssigkeitschromatographievorrichtung, einer Ultrahochleistungsflüssigkeitschromatographievorrichtung, einer Massenspektrometrievorrichtung und einer Glykananalysevorrichtung oder einer Kombination davon besteht.

8. Verfahren nach Anspruch 6 oder 7, ferner umfassend vor (e) das Bewegen des Mehrwegeventils über die Steuervorrichtung in die dritte Position und das Bewegen eines Denaturierungsreagens über die Steuervorrichtung in die Reaktionskammer.

9. Verfahren nach Anspruch 8, wobei das Bewegen des Denaturierungsreagens in die Reaktionskammer umfasst: Bewegen des Denaturierungsreagens über die Steuervorrichtung von einer Denaturierungspufferquelle zu der ersten Haltespule über eine Pumpe und Bewegen des Denaturierungsreagens über die Steuervorrichtung von der ersten Haltespule zu der Reaktionskammer über den dritten Anschluss des Mehrwegeventils.

10. Verfahren nach Anspruch 8 oder 9, wobei (i) ferner das Bewegen des Denaturierungsreagens von der Reaktionskammer zu der ersten Haltespule über den dritten Anschluss umfasst, und wobei (j) ferner das Bewegen des Denaturierungsreagens von der ersten Haltespule zu der Entsalzungssäule umfasst, und wobei, wenn die denaturierten, reduzierten und alkylierten Polypeptide auf die Entsalzungssäule aufgebracht werden, die denaturierten, reduzierten und alkylierten Polypeptide weiter von dem Denaturierungsreagens getrennt werden.

11. Geschlossenes System für die Durchführung eines Online-Echtzeit-Assays, wobei das System umfasst:

eine erste Haltespule (108), die angeordnet ist, um eine Probe eines Polypeptide enthaltenden Produkts aufzunehmen;
ein Mehrwegeventil (104), das mit der Haltespule fluidisch gekoppelt ist und sich stromabwärts von dieser befindet;
eine polypeptidbindende Säule (112), die mit dem Mehrwegeventil fluidisch gekoppelt ist und angeordnet ist, um die Probe von der ersten Haltespule über einen ersten Anschluss des Mehrwegeventils aufzunehmen, wobei die polypeptidbindende Säule dazu konfiguriert ist, die Polypeptide aus der Probe zu binden,
eine Pufferquelle, die mit dem Mehrwegeventil fluidisch gekoppelt ist und angeordnet ist, einer zweiten Haltespule, die sich stromabwärts der polypeptidbindenden Säule befindet, Elutionspufferlösung zuzuführen, sodass die Elutionspufferlösung angepasst ist, um im Wesentlichen alle Polypeptide von der polypeptidbindenden Säule zu eluieren;
eine Reaktionskammer (120), die mit dem Mehrwegeventil fluidisch gekoppelt ist und stromabwärts der polypeptidbindenden Säule angeordnet ist, wobei die Reaktionskammer angepasst ist, um eine Mischung von der polypeptidbindenden Säule über einen zweiten Anschluss des Mehrwegeventils aufzunehmen, wobei die Mischung die Elutionspufferlösung und die eluierten Polypeptide umfasst, wobei die Polypeptide der Mischung in der Reaktionskammer denaturiert werden, wobei die Reaktionskammer angeordnet ist, um über die erste Haltespule und einen dritten Anschluss des Mehrwegeventils ein reduzierendes Reagenz aufzunehmen, das Disulfidbindungsvernetzungen spaltet, wobei das reduzierende Reagens die denaturierten Polypeptide reduziert, und wobei die Reaktionskammer ferner angeordnet ist, um über die erste Haltespule und den dritten Anschluss des Mehrwegeventils ein Alkylierungsreagens aufzunehmen, das Sulfhydrole alkyliert, wobei das Alkylierungsreagens die denaturierten und reduzierten Polypeptide in der Reaktionskammer alkyliert;
eine Entsalzungssäule (124), die mit dem Mehrwegeventil fluidisch gekoppelt ist und angeordnet ist, um die denaturierten, reduzierten und alkylierten Polypeptide, die Elutionspufferlösung und das Alkylierungsreagens aus der Reaktionskammer aufzunehmen, wobei die Entsalzungssäule dazu konfiguriert ist, die denaturierten, reduzierten und alkylierten Polypeptide von der Elutionspufferlösung, dem Reduktionsreagens und dem Alkylierungsreagens zu trennen; und
eine proteolytische Enzymsäule (128), die mit der zweiten Polypeptidsäule fluidisch gekoppelt ist und stromabwärts von dieser angeordnet ist, um die abgetrennten Polypeptide von der Entsalzungssäule zu erhalten, wobei die proteolytische Enzymsäule dazu konfiguriert ist, die entsalzten Polypeptide zu verdauen.

12. Geschlossenes System für die Durchführung eines Echtzeit-Assays, wobei das System umfasst:

ein Mehrwegeventil (104);
eine erste Haltespule (108) stromaufwärts des Mehrwegeventils;
eine polypeptidbindende Säule (112), die mit einem ersten Anschluss des Mehrwegeventils fluidisch gekoppelt ist und stromabwärts von diesem angeordnet ist;
eine zweite Haltespule (116), die mit der polypeptidbindende Säule fluidisch gekoppelt ist und stromabwärts von dieser ist;
eine Reaktionskammer (120), die mit dem zweiten und dem dritten Anschluss des Mehrwegeventils fluidisch gekoppelt ist und stromabwärts von diesen ist;
eine Entsalzungssäule (124), die mit einem vierten Anschluss des Mehrwegeventils fluidisch gekoppelt ist und stromabwärts von diesem ist;
eine proteolytische Enzymsäule (128) stromabwärts der Entsalzungssäule; und eine Steuervorrichtung (132), die kommunikativ mit dem Mehrwegeventil gekoppelt ist und einen Speicher, einen Prozessor und eine Logik umfasst, die in dem Speicher gespeichert ist und von dem Prozessor ausgeführt werden kann, zum:

(a) Bewegen einer Probe eines Produkts, das Polypeptide enthält, zu der ersten Haltespule;
(b) Positionieren des Mehrwegeventils in einer ersten Position, in der die erste Haltespule über den ersten Anschluss des Mehrwegeventils mit der polypeptidbindenden Säule verbunden ist, sodass die Probe zu der ersten Säule fließt, wodurch Polypeptide in der Probe an die polypeptidbindende Säule binden;
(c) wenn sich das Mehrwegeventil in der ersten Position befindet, Bewegen einer Elutionspufferlösung von einer Quelle für Elutionspufferlösung über die polypeptidbindende Säule zu der zweiten Haltespule, sodass die Elutionspufferlösung im Wesentlichen alle an die polypeptidbindende Säule gebundenen Polypeptide eluiert;
(d) Bewegen des Mehrwegeventils in eine zweite Position, in der die zweite Haltespule über den zweiten Anschluss des Mehrwegeventils mit der Reaktionskammer verbunden ist;
(e) wenn sich das Mehrwegeventil in der zweiten Position befindet, Bewegen einer Elutions/Polypeptidmischung, die die Elutionspufferlösung und die eluierten Polypeptide umfasst, in die Reaktionskammer, wodurch die Polypeptide in der Elutions/Polypeptidmischung denaturiert werden;
(f) Bewegen des Mehrwegeventils in eine dritte Position, in der die erste Haltespule über den dritten Anschluss des Mehrwegeventils mit der Reaktionskammer verbunden ist;
(g) nach (f), Bewegen eines reduzierenden Reagens, das Disulfidbindungen spaltet, zu der ersten Haltespule und Bewegen des reduzierenden Reagens von der ersten Haltespule zu der Reaktionskammer über den dritten Anschluss des Mehranschlussventils, wobei dadurch die denaturierten Polypeptide reduziert werden;
(h) nach (g), Bewegen eines Alkylierungsreagens, das Sulfhydryle alkyliert, zu der ersten Haltespule und Bewegen des Alkylierungsreagens von der ersten Haltespule über die dritte Öffnung zu der Reaktionskammer, wobei dadurch die denaturierten und reduzierten Polypeptide alkyliert werden;
(i) Bewegen der denaturierten, reduzierten und alkylierten Polypeptide, der Elutionspufferlösung, des reduzierenden Reagens und des Alkylierungsreagens von der Reaktionskammer zu der ersten Haltespule über den dritten Anschluss;
(j) Bewegen des Mehrwegeventils in eine vierte Position, in der die erste Haltespule über den vierten Anschluss des Mehrwegeventils mit der Entsalzungssäule verbunden ist, und, wenn sich das Mehrwegeventil in der vierten Position befindet, Bewegen der denaturierten, reduzierten und alkylierten Polypeptide, der Elutionspufferlösung, des Reduktionsreagens, und des Alkylierungsreagens von der ersten Haltespule zu der Entsalzungssäule, wodurch die denaturierten, reduzierten und alkylierten Polypeptide entsalzt werden;
(k) Bewegen der entsalzten Polypeptide zu der proteolytischen Enzymsäule, wodurch die entsalzten Polypeptide verdaut werden; und
(l) Passieren der verdauten Polypeptide zu einer Glykananalysevorrichtung, wodurch die verdauten Polypeptide getrennt und quantifiziert werden.

13. Verfahren nach einem der Ansprüche 1-10 oder System nach Anspruch 11-12, wobei das Polypeptid des Produkts ein therapeutisches Polypeptid ist;
optional wobei das therapeutische Polypeptid aus der Gruppe ausgewählt ist, die aus einem Antikörper oder einem antigenbindenden Fragment davon, einem Derivat eines Antikörpers oder Antikörperfragments und einem Fusionspolypeptid besteht.

14. Verfahren oder System nach Anspruch 13, wobei der Antikörper optional aus der Gruppe ausgewählt ist, bestehend aus Infliximab, Bevacizumab, Ranibizumab, Cetuximab, Ranibizumab, Palivizumab, Abagovomab, Abciximab, Actoxumab, Adalimumab, Afelimomab, Afutuzumab, Alacizumab, Alacizumabpegol, Ald518, Alemtuzumab, Alirocumab, Alemtuzumab, Altumomab, Amatuximab, Anatumomab Mafenatox, Anrukinzumab, Apolizumab, Arcitumomab, Aselizumab, Altinumab, Atlizumab, Atorolimiumab, Tocilizumab, Bapineuzumab, Basiliximab, Bavituximab, Bectumomab, Belimumab, Benralizumab, Bertilimumab, Besilesomab, Bevacizumab, Bezlotoxumab, Biciromab, Bivatuzumab, Bivatuzumab Mertansin, Blinatumomab, Blosozumab, Brentuximabvedotin, Briakinumab, Brodalumab, Canakinumab, Cantuzumabmertansin, Cantuzumabmertansin, Caplacizumab, Capromabpendetid, Carlumab, Catumaxomab, cc49, Cedelizumab, Certolizumabpegol, Cetuximab, Citatuzumabbogatox, Cixutumumab, Clazakizumab, Clenoliximab, Clivatuzumabtetraxetan, Conatumumab, Crenezumab, cr6261, Dacetuzumab, Daclizumab, Dalotuzumab, Daratumumab, Demcizumab, Denosumab, Detumomab, Dorlimomabaritox, Drozitumab, Duligotumab, Dupilumab, Ecromeximab, Eculizumab, Edobacomab, Edrecolomab, Efalizumab, Efungumab, Elotuzumab, Elsilimomab, Enavatuzumab, Enlimomabpegol, Enokizumab, Enokizumab, Enoticumab, Enoticumab, Ensituximab, Epitumomabcituxetan, Epratuzumab, Erlizumab, Ertumaxomab, Etaracizumab, Etrolizumab, Exbivirumab, Exbivirumab, Fanolesomab, Faralimomab, Farletuzumab, Fasinumab, fbta05, Felvizumab, Fezakinumab, Ficlatuzumab, Figitumumab, Flanvotumab, Fontolizumab, Foralumab, Foravirumab, Fresolimumab, Fulranumab, Futuximab, Ga-

liximab, Ganitumab, Gantenerumab, Gavilimomab, Gemtuzumabozogamicin, Gevokizumab, Girentuximab, Glembatumumabvedotin, Golimumab, Gomiliximab, gs6624, Ibalizumab, Ibritumomabtiuxetan, Icrucumab, Igovomab, Imciromab, Imgatuzumab, Inclacumab, Indatuximabravtansin, Infliximab, Intetumumab, Inolimomab, Inotuzumabozogamicin, Ipilimumab, Iratumumab, Itolizumab, Ixekizumab, Keliximab, Labetuzumab, Lebrikizumab, Lemalesomab, Lerdelimumab, Lexatumumab, Libivirumab, Ligelizumab, Lintuzumab, Lirilumab, Lorvotuzumabmertansin, Lucatumumab, Lumiliximab, Mapatumumab, Maslimomab, Mavrilimumab, Matuzumab, Mepolizumab, Metelimumab, Milatuzumab, Minretumomab, Mitumomab, Mogamulizumab, Morolimumab, Motavizumab, Moxetumomabpasudotox, Muromonab-cd3, Nacolomabtafenatox, Namilumab, Naptumomabestafenatox, Namatumab, Natalizumab, Nebacumab, Necitumumab, Nerelimomab, Nesvacumab, Nimotuzumab, Nivolumab, Nofetumomabmerpentan, Ocaratuzumab, Ocrelizumab, Odulimomab, Ofatumumab, Olaratumab, Olokizumab, Omalizumab, Onartuzumab, Oportuzumabmonatox, Oregovomab, Orticumab, Otelixizumab, Oxelumab, Ozanezumab, Ozoralizumab, Pagibaximab, Palivizumab, Panitumumab, Panobacumab, Parsatuzumab, Pascolizumab, Pateclizumab, Patritumab, Pemtumomab, Perakizumab, Pertuzumab, Pexelizumab, Pidilizumab, Pintumomab, Placulumab, Ponezumab, Priliximab, Pritumumab, PRO 140, Quilizumab, Racotumomab, Radretumab, Rafivirumab, Ramucirumab, Ranibizumab, Raxibacumab, Regavirumab, Reslizumab, Rilotumumab, Rituximab, Robatumumab, Roledumab, Romosozumab, Rontalizumab, Rovelizumab, Ruplizumab, Samalizumab, Sarilumab, Satumomabpendetid, Secukinumab, Sevirumab, Sibrotuzumab, Sifalimumab, Siltuximab, Simtuzumab, Siplizumab, Sirukumab, Solanezumab, Solitomab, Sonepcizumab, Sontuzumab, Stamulumab, Sulesomab, Suvizumab, Tabalumab, Tacatuzumabtetraxetan, Tadocizumab, Talizumab, Tanezumab, Taplitumomabpaptox, Tefibazumab, Telimomabaritox, Tenatumomab, Tefibazumab, Telimomabaritox, Tenatumomab, Teneliximab, Teplizumab, Teprotumumab, Tezepelumab, TGN1412, Tremelimumab, Ticilimumab, Tildrakizumab, Tigatuzumab, TNX-650, Tocilizumab, Toralizumab, Tositumomab, Tralokinumab, Trastuzumab, TRBS07, Tregalizumab, Tremelimumab, Tucotuzumabcelmoleukin, Tuvirumab, Ublituximab, Urelumab, Urtoxazumab, Ustekinumab, Vapaliximab, Vatelizumab, Vedolizumab, Veltuzumab, Vepalimomab, Vesencumab, Visilizumab, Volociximab, Vorsetuzumabmafodotin, Votumumab, Zalutumumab, Zanolimumab, Zatuximab, Ziralimumab, Zolimomabaritox, und den in Tabelle 1 gezeigten Antikörpern.

15. Verfahren oder System nach Anspruch 13, wobei das therapeutische Polypeptid ein Polypeptid ist, das aus der Gruppe ausgewählt ist, bestehend aus einem Glykoprotein, einem CD-Polypeptid, einem HER-Rezeptor-Polypeptid, einem Zelladhäsionspolypeptid, einem Wachstumsfaktorpolypeptid, einem Insulinpolypeptid, einem mit Insulin verwandten Polypeptid, einem Gerinnungspolypeptid, ein gerinnungsbezogenem Polypeptid, Albumin, IgE, einem Blutgruppenantigen, einem koloniestimulierenden Faktor, einem Rezeptor, einem neurotrophen Faktor, einem Interferon, einem Interleukin, einem viralen Antigen, einem Lipoprotein, Calcitonin, Glukagon, atrialem natriuretischem Faktor, Lungensurfactant, Tumor-Nekrose-Faktor-alpha und -beta, Enkephalinase, Maus-Gonadotropin-assoziiertem Peptid, DNAse, Inhibin, Activing, einem Integrin, Protein A, Protein D, einem Rheumafaktor, einem Immuntoxin, einem knochenmorphogenetischen Protein, einer Superoxid-Dismutase, einem Oberflächenmembranpolypeptid, einem Zerfallsbeschleunigungsfaktor, einer AIDS-Hülle, einem Transportpolypeptid, einem Homing-Rezeptor, einem Addressin, einem regulatorischen Polypeptid, einem Immunoadhesin, einem Myostatin, einem TALL-Polypeptid, einem Amyloidpolypeptid, einem thymischen stromalen Lymphopoietin, einem RANK-Liganden, einem c-kit-Polypeptid, einem TNF-Rezeptor und einem Angiopoietin, und biologisch aktiven Fragmenten, Analoga oder Varianten davon.

Revendications

1. Procédé permettant de conduire un dosage en temps réel, le procédé comprenant les étapes consistant à :

(a) apporter un échantillon d'un produit contenant des polypeptides à une colonne de liaison de polypeptides (112) par l'intermédiaire d'un premier serpentin de retenue (108) ;
(b) lier les polypeptides de l'échantillon à la colonne de liaison de polypeptides, séparant ainsi les polypeptides de l'échantillon du reste de l'échantillon ;
(c) apporter une solution de tampon d'élution provenant d'une source de tampon à la colonne de liaison de polypeptides, par l'intermédiaire du premier serpentin de retenue et lui faire traverser un deuxième serpentin de retenue (116) en aval de la colonne de liaison de polypeptides, éluant ainsi les polypeptides liés à la colonne de liaison de polypeptides et apportant un mélange élution/polypeptides comprenant la solution de tampon d'élution et les polypeptides élués au deuxième serpentin de retenue ;
(d) apporter le mélange d'élution/polypeptides provenant du deuxième serpentin de retenue à une chambre de réaction (120) ;
(e) incuber les polypeptides du mélange d'élution/polypeptides dans la chambre de réaction, produisant ainsi

des polypeptides dénaturés ;
(f) apporter un réactif réducteur, qui clive les réticulations de liaison disulfure, à la chambre de réaction par l'intermédiaire du premier serpentin de retenue après (e) ;
(g) incuber les polypeptides dénaturés avec le réactif réducteur dans la chambre de réaction, produisant ainsi des polypeptides dénaturés et réduits ;
(h) apporter un réactif d'alkylation, qui alkyle les sulfhydryles, à la chambre de réaction après (g) ;
(i) incuber les polypeptides dénaturés et réduits avec le réactif d'alkylation dans la chambre de réaction, alkylant ainsi les polypeptides dénaturés et réduits ;
(j) apporter les polypeptides dénaturés, réduits et alkylés, la solution de tampon d'élution, le réactif réducteur et le réactif d'alkylation provenant de la chambre de réaction à une colonne de dessalage (124) par l'intermédiaire du premier serpentin de retenue, la colonne de dessalage étant équilibrée à l'aide d'un tampon de protéolyse ;
(k) appliquer les polypeptides dénaturés, réduits et alkylés à la colonne de dessalage, séparant ainsi les polypeptides dénaturés, réduits et alkylés des réactifs réducteurs et d'alkylations, et produisant ainsi des polypeptides dessalés ;
(l) apporter les polypeptides dessalés à une colonne d'enzymes protéolytiques (128) en aval de la colonne de dessalage ;
(m) digérer les polypeptides dessalés dans la colonne d'enzymes protéolytiques, produisant ainsi des polypeptides digérés ; et
(n) apporter les polypeptides digérés à un dispositif analytique (156) pour l'analyse des polypeptides digérés.

2. Procédé selon la revendication 1, dans lequel un ou plusieurs des points (a), (c), (d), (f), (h), (i), (l) et (n) sont réalisés de façon automatique ; et/ou dans lequel les points (a) à (n) sont réalisés dans un système fermé.

3. Procédé selon les revendications 1 ou 2, dans lequel (a) comprend le positionnement d'une vanne multivoie (104) dans une première position dans laquelle le premier serpentin de retenue et la colonne de liaison de polypeptides sont couplés fluidiquement par l'intermédiaire d'une première voie de la vanne multivoie ;
le point (d) comprenant éventuellement le positionnement de la vanne multivoie dans une deuxième position dans laquelle le deuxième serpentin de retenue et la chambre de réaction sont fluidiquement couplés par l'intermédiaire d'une deuxième voie de la vanne multivoie.

4. Procédé selon la revendication 3, dans lequel les points (f) et (h) comprennent chacun le positionnement de la vanne multivoie dans une troisième position dans laquelle le premier serpentin de retenue et la chambre de réaction sont couplés fluidiquement par l'intermédiaire d'une troisième voie de la vanne multivoie ;
le point (j) comprenant éventuellement :

l'apport des polypeptides dénaturés, réduits et alkylés, de la solution de tampon d'élution, du réactif réducteur et du réactif d'alkylation provenant de la chambre de réaction à la colonne de dessalage par l'intermédiaire de la troisième voie de la vanne multivoie ; et
le positionnement de la vanne multivoie dans une quatrième position dans laquelle le premier serpentin de retenue et la colonne de dessalage sont fluidiquement couplés par l'intermédiaire d'une quatrième voie de la vanne multivoie.

5. Procédé selon l'une quelconque des revendications 1 à 4, comprenant en outre :

avant ou pendant (d), le passage d'une première vanne (256) couplée fluidiquement à la chambre de réaction, et située en aval de celle-ci, dans une première position dans laquelle la première vanne dirige le contenu, reçu du premier serpentin de retenue en une quantité supérieure au volume de la chambre de réaction, vers une chambre de rejet, ledit procédé comprenant éventuellement en outre, après (i), le passage de la première vanne, de la première position, dans une deuxième position dans laquelle la première vanne dirige de l'air dans la chambre de réaction ; et/ou
après (k) et avant (l), (o) le passage d'une deuxième vanne (280) couplée fluidiquement aux colonnes de dessalage et d'enzymes protéolytiques, et située entre celles-ci, d'une première position dans laquelle la deuxième vanne dirige le contenu reçu de la colonne de dessalage vers une chambre de rejet, dans une deuxième position dans laquelle la deuxième vanne dirige le contenu reçu de la colonne de dessalage vers la colonne d'enzymes protéolytiques ; et/ou
le point (a) comprend l'apport de l'échantillon du produit, provenant d'un récipient contenant les polypeptides, à la colonne de liaison de polypeptides par l'intermédiaire du premier serpentin de retenue ; et/ou
avant (d), le remplissage au moins partiel de la chambre de réaction avec un réactif dénaturant, le point (e)

comprenant l'incubation des polypeptides du mélange d'élution/polypeptides avec le réactif dénaturant dans la chambre de réaction, l'opération de remplissage au moins partiel de la chambre de réaction avec le réactif dénaturant comprenant éventuellement l'apport du réactif dénaturant provenant d'une source de tampon dénaturant à la chambre de réaction par l'intermédiaire du premier serpentin de retenue, ou le point (j) comprenant éventuellement en outre l'apport du réactif dénaturant, provenant de la chambre de réaction, à la colonne de dessalage par l'intermédiaire du premier serpentin de retenue, et le point (k) séparant en outre les polypeptides dénaturés, réduits et alkylés du réactif dénaturant ; et/ou
l'application de chaleur à la chambre de réaction.

6. Procédé permettant de conduire un dosage en temps réel à l'aide d'un système fermé comprenant une vanne multivoie (104), un premier serpentin de retenue (108) en amont de la vanne multivoie, une colonne de liaison de polypeptides (112) couplée fluidiquement à une première voie de la vanne multivoie et en aval de celle-ci, un deuxième serpentin de retenue (116) couplé fluidiquement à la colonne de liaison de polypeptides et en aval de celle-ci, une chambre de réaction (120) couplée fluidiquement à des deuxième et troisième voies de la vanne multivoie et en aval de celles-ci, une colonne de dessalage couplée fluidiquement à une quatrième voie de la vanne multivoie et en aval de celle-ci, et une colonne d'enzymes protéolytiques (128) en aval de la colonne de dessalage, le procédé comprenant :

(a) l'apport, par l'intermédiaire d'une unité de commande couplée de manière communicative au système fermé, d'un échantillon d'un produit comprenant des polypeptides au premier serpentin de retenue ;
(b) le positionnement, par l'intermédiaire de l'unité de commande, de la vanne multivoie dans une première position dans laquelle le premier serpentin de retenue est relié à la colonne de liaison de polypeptides par l'intermédiaire de la première voie de la vanne multivoie, de sorte que l'échantillon s'écoule vers la première colonne, moyennant quoi les polypeptides de l'échantillon se lient à la colonne de liaison de polypeptides ;
(c) lorsque la vanne multivoie est dans la première position, l'apport, par l'intermédiaire de l'unité de commande, d'une solution de tampon d'élution, provenant d'une source de solution de tampon d'élution, au deuxième serpentin de retenue par l'intermédiaire de la colonne de liaison de polypeptides, de sorte que la solution de tampon d'élution élue la quasi-totalité des polypeptides liés à la colonne de liaison de polypeptides ;
(d) le passage, par l'intermédiaire de l'unité de commande, de la vanne multivoie dans une deuxième position dans laquelle le deuxième serpentin de retenue est relié à une chambre de réaction par l'intermédiaire de la deuxième voie de la vanne multivoie ;
(e) lorsque la vanne multivoie est dans la deuxième position, l'apport, par l'intermédiaire de l'unité de commande, d'un mélange d'élution/polypeptides, comprenant la solution de tampon d'élution et les polypeptides élués, à la chambre de réaction, permettant ainsi de dénaturer les polypeptides du mélange d'élution/polypeptides ;
(f) le passage, par l'intermédiaire de l'unité de commande, de la vanne multivoie dans une troisième position dans laquelle le premier serpentin de retenue est relié à une chambre de réaction par l'intermédiaire de la troisième voie de la vanne multivoie ;
(g) après (f), l'apport, par l'intermédiaire de l'unité de commande, d'un réactif réducteur, qui clive les réticulations de liaison disulfure, au premier serpentin de retenue, et l'apport, par l'intermédiaire de l'unité de commande, du réactif réducteur provenant du premier serpentin de retenue à la chambre de réaction par l'intermédiaire de la troisième voie de la vanne multivoie, réduisant ainsi les polypeptides dénaturés ;
(h) après (g), l'apport, par l'intermédiaire de l'unité de commande, d'un réactif d'alkylation, qui alkyle les groupes sulfhydryles, au premier serpentin de retenue, et l'apport, par l'intermédiaire de l'unité de commande, du réactif d'alkylation provenant du premier serpentin de retenue à la chambre de réaction par l'intermédiaire de la troisième voie, alkylant ainsi les polypeptides dénaturés et réduits ;
(i) l'apport, par l'intermédiaire de l'unité de commande, des polypeptides alkylés, de la solution de tampon d'élution, du réactif réducteur et du réactif d'alkylation provenant de la chambre de réaction au premier serpentin de retenue par l'intermédiaire de la troisième voie ;
(j) le passage, par l'intermédiaire de l'unité de commande, de la vanne multivoie dans une quatrième position dans laquelle le premier serpentin de retenue est relié à la colonne de dessalage par l'intermédiaire de la quatrième voie de la vanne multivoie et, lorsque la vanne multivoie est dans la quatrième position, l'apport des polypeptides alkylés, de la solution de tampon d'élution, du réactif réducteur et du réactif d'alkylation provenant du premier serpentin de retenue à la colonne de dessalage, permettant ainsi aux polypeptides dénaturés, réduits et alkylés d'être appliqués à la colonne de dessalage, séparant ainsi les polypeptides dénaturés, réduits et alkylés des réactifs réducteurs et d'alkylation, produisant ainsi des polypeptides dessalés ;
(k) l'apport, par l'intermédiaire de l'unité de commande, des polypeptides dessalés à la colonne d'enzymes protéolytiques, permettant ainsi la digestion des polypeptides dessalés ; et
(l) le passage, par l'intermédiaire de l'unité de commande, des polypeptides digérés vers un appareil d'analyse

(156), permettant ainsi l'analyse des polypeptides digérés.

7. Procédé selon la revendication 6, dans lequel le point (a) comprend l'apport, par l'intermédiaire de l'unité de commande, de l'échantillon provenant d'un récipient contenant les polypeptides au premier serpentin de retenue, et/ou dans lequel le point (k) comprend le passage des polypeptides digérés vers un appareil d'analyse sélectionné dans le groupe constitué de : un dispositif de chromatographie liquide, un dispositif de chromatographie liquide à haute performance, un dispositif de chromatographie liquide à ultra-haute performance, un dispositif de spectrométrie de masse et un dispositif d'analyse des glycanes, ou une combinaison de ceux-ci.

8. Procédé selon la revendication 6 ou 7, comprenant en outre, avant (e), le passage, par l'intermédiaire de l'unité de commande, de la vanne multivoie dans la troisième position, et l'apport, par l'intermédiaire de l'unité de commande, d'un réactif dénaturant à la chambre de réaction.

9. Procédé selon la revendication 8, dans lequel l'apport du réactif dénaturant à la chambre de réaction comprend : l'apport, par l'intermédiaire de l'unité de commande, du réactif dénaturant provenant d'une source de tampon dénaturant au premier serpentin de retenue par l'intermédiaire d'une pompe, et l'apport, par l'intermédiaire de l'unité de commande, du réactif dénaturant provenant du premier serpentin de retenue à la chambre de réaction par l'intermédiaire de la troisième voie de la vanne multivoie.

10. Procédé selon la revendication 8 ou 9, dans lequel le point (i) comprend en outre l'apport du réactif dénaturant provenant de la chambre de réaction au premier serpentin de retenue par l'intermédiaire de la troisième voie, et dans lequel le point (j) comprend en outre l'apport du réactif dénaturant provenant du premier serpentin de retenue à la colonne de dessalage, permettant ainsi, lorsque les polypeptides dénaturés, réduits et alkylés sont appliqués à la colonne de dessalage, de séparer davantage les polypeptides dénaturés, réduits et alkylés du réactif dénaturant.

11. Système fermé permettant de conduire un dosage en ligne et en temps réel, le système comprenant :

un premier serpentin de retenue (108) agencé fluidiquement pour recevoir un échantillon d'un produit contenant des polypeptides ;
une vanne multivoie (104) couplée fluidiquement au premier serpentin de retenue et située en aval de celui-ci ;
une colonne de liaison de polypeptides (112) couplée fluidiquement à la vanne multivoie et agencée pour recevoir l'échantillon provenant du premier serpentin de retenue par l'intermédiaire d'une première voie de la vanne multivoie, la colonne de liaison de polypeptides étant conçue pour lier les polypeptides de l'échantillon,
une source de tampon couplée fluidiquement à la vanne multivoie et agencée pour fournir une solution de tampon d'élution à un deuxième serpentin de retenue situé en aval de la colonne de liaison de polypeptides, de telle sorte que la solution de tampon d'élution est adaptée à éluer la quasi-totalité des polypeptides de la colonne de liaison de polypeptides ;
une chambre de réaction (120) couplée fluidiquement à la vanne multivoie et agencée en aval de la colonne de liaison de polypeptides, la chambre de réaction étant adaptée à recevoir un mélange provenant de la colonne de liaison de polypeptides par l'intermédiaire d'une deuxième voie de la vanne multivoie, le mélange comprenant la solution de tampon d'élution et les polypeptides élués, lesdits polypeptides du mélange étant dénaturés dans la chambre de réaction, ladite chambre de réaction étant agencée pour recevoir un réactif réducteur, qui clive les réticulations de liaison disulfure, par l'intermédiaire du premier serpentin de retenue et d'une troisième voie de la vanne multivoie, le réactif réducteur réduisant les polypeptides dénaturés, et ladite chambre de réaction étant en outre agencée pour recevoir un réactif d'alkylation, qui alkyle les sulfhydryles, par l'intermédiaire du premier serpentin de retenue et de la troisième voie de la vanne multivoie, ledit réactif d'alkylation entraînant l'alkylation des polypeptides dénaturés et réduits dans la chambre de réaction ;
une colonne de dessalage (124) couplée fluidiquement à la vanne multivoie et agencée pour recevoir les polypeptides dénaturés, réduits et alkylés, la solution de tampon d'élution et le réactif d'alkylation provenant de la chambre de réaction, la colonne de dessalage étant conçue pour séparer les polypeptides dénaturés, réduits et alkylés de la solution de tampon d'élution, du réactif réducteur et du réactif d'alkylation ; et
une colonne d'enzymes protéolytiques (128) couplée fluidiquement à la deuxième colonne de polypeptides pour obtenir les polypeptides séparés de la colonne de dessalage, la colonne d'enzymes protéolytiques étant conçue pour digérer les polypeptides dessalés.

12. Système fermé permettant de conduire un dosage en temps réel, le système comprenant :

une vanne multivoie (104) ;

un premier serpentin de retenue (108) en amont de la vanne multivoie ;
une colonne de liaison de polypeptides (112) couplée fluidiquement à une première voie de la vanne multivoie et en aval de celle-ci ;
un deuxième serpentin de retenue (116) couplé fluidiquement à la colonne de liaison de polypeptides et en aval de celle-ci ;
une chambre de réaction (120) couplée fluidiquement aux deuxième et troisième voies de la vanne multivoie et en aval de celles-ci ;
une colonne de dessalage (124) couplée fluidiquement à une quatrième voie de la vanne multivoie et en aval de celle-ci ;
une colonne d'enzymes protéolytiques (128) en aval de la colonne de dessalage ; et
une unité de commande (132) couplée de manière communicative à la vanne multivoie et comprenant une mémoire, un processeur et une logique stockée sur la mémoire et exécutable par le processeur pour :

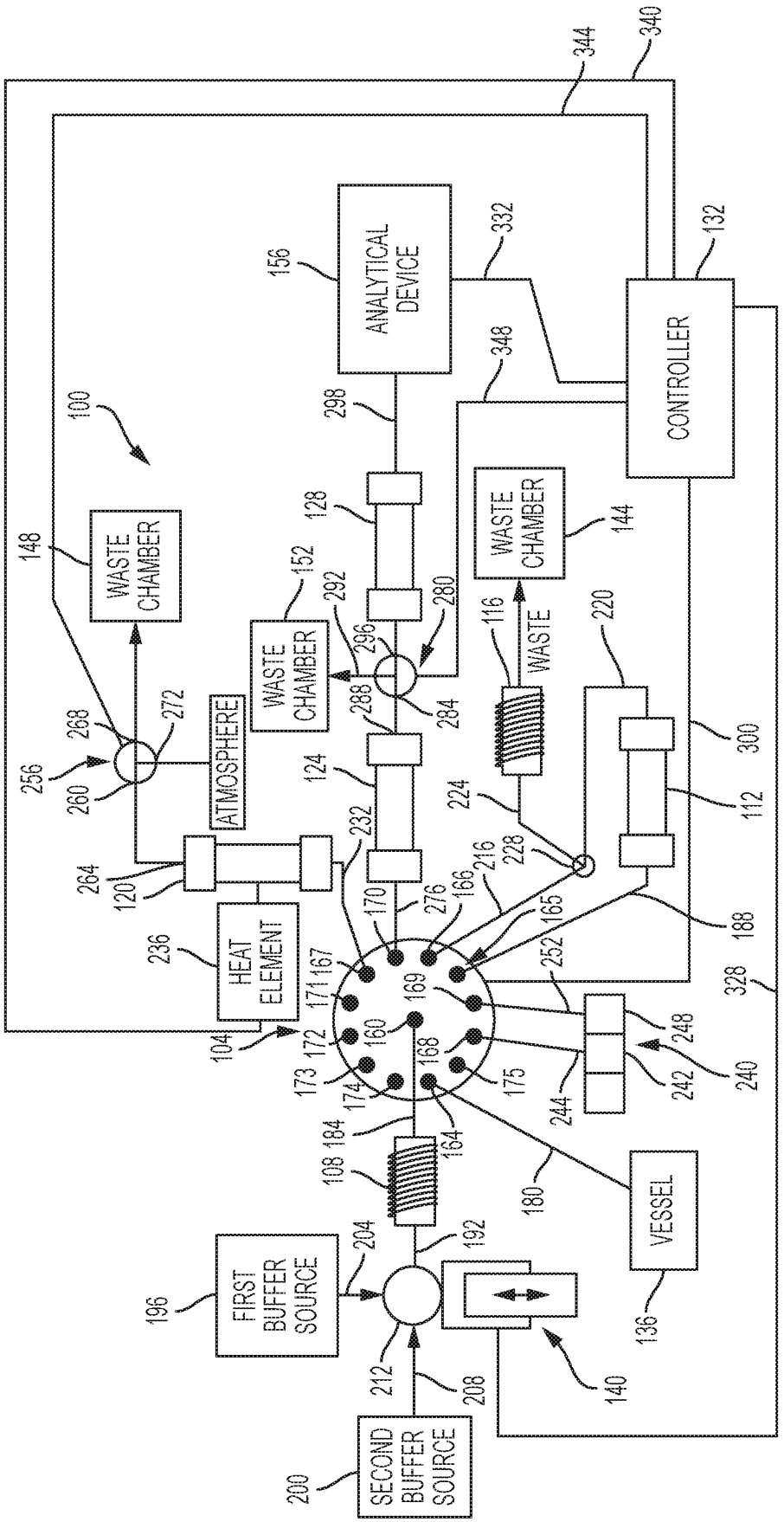
(a) apporter un échantillon d'un produit contenant des polypeptides au premier serpentin de retenue ;
(b) faire passer la vanne multivoie dans une première position dans laquelle le premier serpentin de retenue est relié à la colonne de liaison de polypeptides par l'intermédiaire de la première voie de la vanne multivoie, de sorte que l'échantillon s'écoule vers la première colonne, moyennant quoi les polypeptides de l'échantillon se lient à la colonne de liaison de polypeptides ;
(c) lorsque la vanne multivoie est dans la première position, apporter une solution de tampon d'élution provenant d'une source de solution de tampon d'élution au deuxième serpentin de retenue par l'intermédiaire de la colonne de liaison de polypeptides, de sorte que la solution de tampon d'élution élue la quasi-totalité des polypeptides liés à la colonne de liaison de polypeptides ;
(d) faire passer la vanne multivoie dans une deuxième position dans laquelle le deuxième serpentin de retenue est relié à une chambre de réaction par l'intermédiaire de la deuxième voie de la vanne multivoie ;
(e) lorsque la vanne multivoie est dans la deuxième position, apporter un mélange d'élution/polypeptides, comprenant la solution de tampon d'élution et les polypeptides élués, à la chambre de réaction, permettant ainsi de dénaturer les polypeptides du mélange d'élution/polypeptides ;
(f) faire passer la vanne multivoie dans une troisième position dans laquelle le premier serpentin de retenue est relié à une chambre de réaction par l'intermédiaire de la troisième voie de la vanne multivoie ;
(g) après (f), apporter un réactif réducteur, qui clive les liaisons disulfure, au premier serpentin de retenue, et apporter le réactif réducteur provenant du premier serpentin de retenue à la chambre de réaction par l'intermédiaire de la troisième voie de la vanne multivoie, réduisant ainsi les polypeptides dénaturés ;
(h) après (g), apporter un réactif d'alkylation, qui alkyle les sulfhydryles, au premier serpentin de retenue, et apporter le réactif d'alkylation provenant du premier serpentin de retenue à la chambre de réaction par l'intermédiaire de la troisième voie, alkylant ainsi les polypeptides dénaturés et réduits ;
(i) apporter les polypeptides dénaturés, réduits et alkylés et la solution de tampon d'élution, le réactif réducteur et le réactif d'alkylation provenant de la chambre de réaction au premier serpentin de retenue par l'intermédiaire de la troisième voie ;
(j) faire passer la vanne multivoie dans une quatrième position dans laquelle le premier serpentin de retenue est relié à la colonne de dessalage par l'intermédiaire de la quatrième voie de la vanne multivoie et, lorsque la vanne multivoie est dans la quatrième position, apporter les polypeptides dénaturés, réduits et alkylés, la solution de tampon d'élution, le réactif réducteur et le réactif d'alkylation provenant du premier serpentin de retenue à la colonne de dessalage, moyennant quoi les polypeptides dénaturés, réduits et alkylés sont dessalés ;
(k) apporter les polypeptides dessalés à la colonne d'enzymes protéolytiques, permettant ainsi la digestion des polypeptides dessalés ; et
(l) faire passer les polypeptides digérés à un dispositif d'analyse des glycanes, permettant de séparer et de quantifier les polypeptides digérés.

13. Procédé selon l'une quelconque des revendications 1 à 10 ou système selon les revendications 11 et 12, dans lequel le polypeptide du produit est un polypeptide thérapeutique ;
ledit polypeptide thérapeutique étant éventuellement sélectionné dans le groupe constitué d'un anticorps ou d'un fragment de liaison à l'antigène de celui-ci, d'un dérivé d'un anticorps ou d'un fragment d'anticorps, et d'un polypeptide de fusion.

14. Procédé ou système selon la revendication 13, dans lequel l'anticorps est sélectionné dans le groupe constitué de : infliximab, bevacizumab, ranibizumab, cetuximab, ranibizumab, palivizumab, abagovomab, abciximab, actoxumab, adalimumab, afelimomab, afutuzumab, alacizumab, alacizumab pegol, ald518, alemtuzumab, alirocumab, alem-

tuzumab, altumomab, amatuximab, anatumomab mafenatox, anrukinzumab, apolizumab, arcitumomab, aselizumab, altinumab, atlizumab, atorolimiumab, tocilizumab, bapineuzumab, basiliximab, bavituximab, bectumomab, belimumab, benralizumab, bertilimumab, besilesomab, bevacizumab, bezlotoxumab, biciromab, bivatuzumab, bivatuzumab mertansine, blinatumomab, blosozumab, brentuximab vedotin, briakinumab, brodalumab, canakinumab, cantuzumab mertansine, cantuzumab mertansine, caplacizumab, capromab pendetide, carlumab, catumaxomab, cc49, cedelizumab, certolizumab pegol, cetuximab, citatuzumab bogatox, cixutumumab, clazakizumab, clenoliximab, clivatuzumab tetraxetan, conatumumab, crenezumab, cr6261, dacetuzumab, daclizumab, dalotuzumab, daratumumab, demcizumab, denosumab, detumomab, dorlimomab aritox, drozitumab, duligotumab, dupilumab, ecromeximab, eculizumab, edobacomab, edrecolomab, efalizumab, efungumab, elotuzumab, elsilimomab, enavatuzumab, enlimomab pegol, enokizumab, enokizumab, enoticumab, enoticumab, ensituximab, epitumomab cituxetan, epratuzumab, erlizumab, ertumaxomab, etaracizumab, etrolizumab, exbivirumab, exbivirumab, fanolesomab, faralimomab, farletuzumab, fasinumab, fbta05, felvizumab, fezakinumab, ficlatuzumab, figitumumab, flanvotumab, fontolizumab, foralumab, foravirumab, fresolimumab, fulranumab, futuximab, galiximab, ganitumab, gantenerumab, gavilimomab, gemtuzumab ozogamicin, gevokizumab, girentuximab, glembatumumab vedotin, golimumab, gomiliximab, gs6624, ibalizumab, ibritumomab tiuxetan, icrucumab, igovomab, imciromab, imgatuzumab, inclacumab, indatuximab ravtansine, infliximab, intetumumab, inolimomab, inotuzumab ozogamicin, ipilimumab, iratumumab, itolizumab, ixekizumab, keliximab, labetuzumab, lebrikizumab, lemalesomab, lerdelimumab, lexatumumab, libivirumab, ligelizumab, lintuzumab, lirilumab, lorvotuzumab mertansine, lucatumumab, lumiliximab, mapatumumab, maslimomab, mavrilimumab, matuzumab, mepolizumab, metelimumab, milatuzumab, minretumomab, mitumomab, mogamulizumab, morolimumab, motavizumab, moxetumomab pasudotox, muromonab-cd3, nacolomab tafenatox, namilumab, naptumomab estafenatox, namatumab, natalizumab, nebacumab, necitumumab, nerelimomab, nesvacumab, nimotuzumab, nivolumab, nofetumomab merpentan, ocaratuzumab, ocrelizumab, odulimomab, ofatumumab, olaratumab, olokizumab, omalizumab, onartuzumab, oportuzumab monatox, oregovomab, orticumab, otelixizumab, oxelumab, ozanezumab, ozoralizumab, pagibaximab, palivizumab, panitumumab, panobacumab, parsatuzumab, pascolizumab, pateclizumab, patritumab, pemtumomab, perakizumab, pertuzumab, pexelizumab, pidilizumab, pintumomab, placulumab, ponezumab, priliximab, pritumumab, PRO 140, quilizumab, racotumomab, radretumab, rafivirumab, ramucirumab, ranibizumab, raxibacumab, regavirumab, reslizumab, rilotumumab, rituximab, robatumumab, roledumab, romosozumab, rontalizumab, rovelizumab, ruplizumab, samalizumab, sarilumab, satumomab pendetide, secukinumab, sevirumab, sibrotuzumab, sifalimumab, siltuximab, simtuzumab, siplizumab, sirukumab, solanezumab, solitomab, sonepcizumab, sontuzumab, stamulumab, sulesomab, suvizumab, tabalumab, tacatuzumab tetraxetan, tadocizumab, talizumab, tanezumab, taplitumomab paptox, tefibazumab, telimomab aritox, tenatumumomab, tefibazumab, telimomab aritox, tenatumomab, teneliximab, teplizumab, teprotumumab, tezepelumab, TGN1412, tremelimumab, ticilimumab, tildrakizumab, tigatuzumab, TNX-650, tocilizumab, toralizumab, tositumomab, tralokinumab, trastuzumab, TRBS07, tregalizumab, tremelimumab, tucotuzumab celmoleukin, tuvirumab, ublituximab, urelumab, urtoxazumab, ustekinumab, vapaliximab, vatelizumab, vedolizumab, veltuzumab, vepalimomab, vesencumab, visilizumab, volociximab, vorsetuzumab mafodotin, votumumab, zalutumumab, zanolimumab, zatuximab, ziralimumab, zolimomab aritox, et les anticorps figurant dans le tableau 1.

15. Procédé ou système selon la revendication 13, dans lequel le polypeptide thérapeutique est un polypeptide sélectionné dans le groupe constitué de : une glycoprotéine, un polypeptide CD, un polypeptide récepteur HER, un polypeptide d'adhésion cellulaire, un polypeptide de facteur de croissance, un polypeptide d'insuline, un polypeptide lié à l'insuline, un polypeptide de coagulation, un polypeptide lié à la coagulation, l'albumine, IgE, un antigène de groupe sanguin, un facteur de stimulation de colonie, un récepteur, un facteur neurotrophique, un interféron, une interleukine, un antigène viral, une lipoprotéine, une calcitonine, le glucagon, un facteur natriurétique auriculaire, un surfactant pulmonaire, un facteur de nécrose tumorale alpha et bêta, une enképhalinase, un peptide associé à la gonadotrophine de souris, une DNAse, une inhibine, une intégrine, une protéine A, une protéine D, un facteur rhumatoïde, une immunotoxine, une protéine morphogénétique osseuse, une superoxyde dismutase, un polypeptide de membrane de surface, un facteur d'accélération de dégradation, une enveloppe du SIDA, un polypeptide de transport, un récepteur d'orientation, une adressine, un polypeptide régulateur, une immunoadhésine, une myostatine, un polypeptide TALL, un polypeptide amyloïde, une lymphopoïétine stromale thymique, un ligand RANK, un polypeptide c-kit, un récepteur TNF et une angiopoïétine, ainsi que les fragments, analogues ou variantes biologiquement actifs de ceux-ci.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WASTE
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ATMOSPHERE
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216
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170
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HEAT ELEMENT
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VESSEL
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FIRST BUFFER SOURCE
212
208
140
136
200
SECOND BUFFER SOURCE

FIG. 1

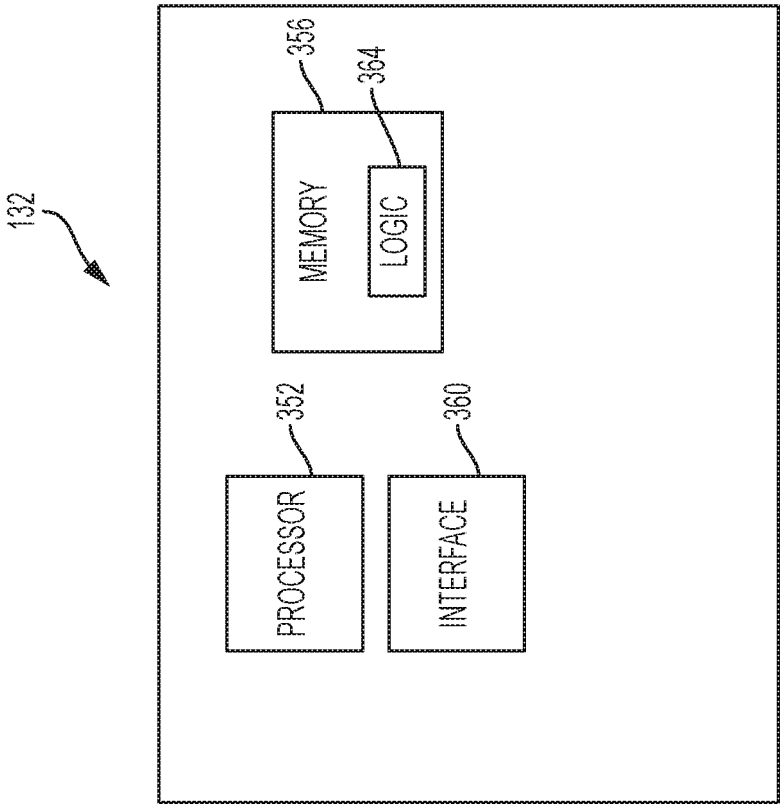
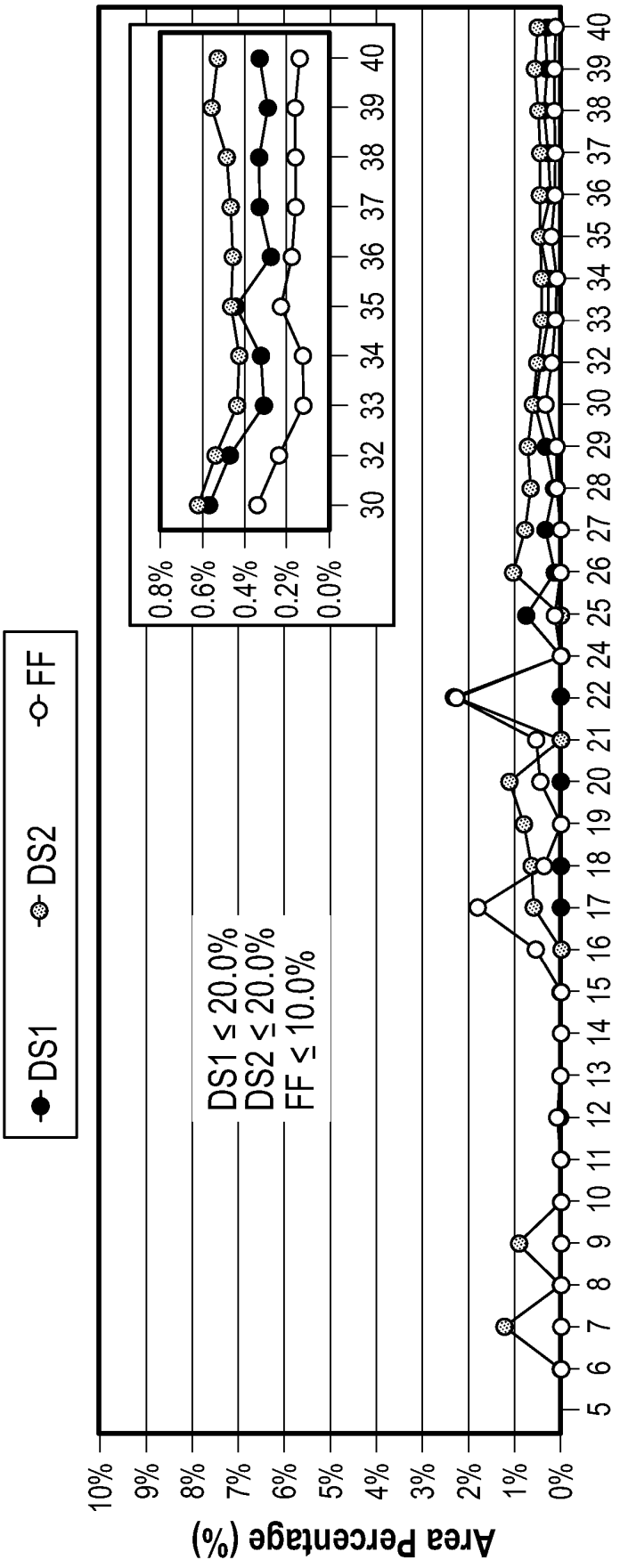


FIG. 2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DS2
FF
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DS2 ≤ 20.0%
FF ≤ 10.0%
Area Percentage (%)
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9%
8%
7%
6%
5%
4%
3%
2%
1%
0%
0.8%
0.6%
0.4%
0.2%
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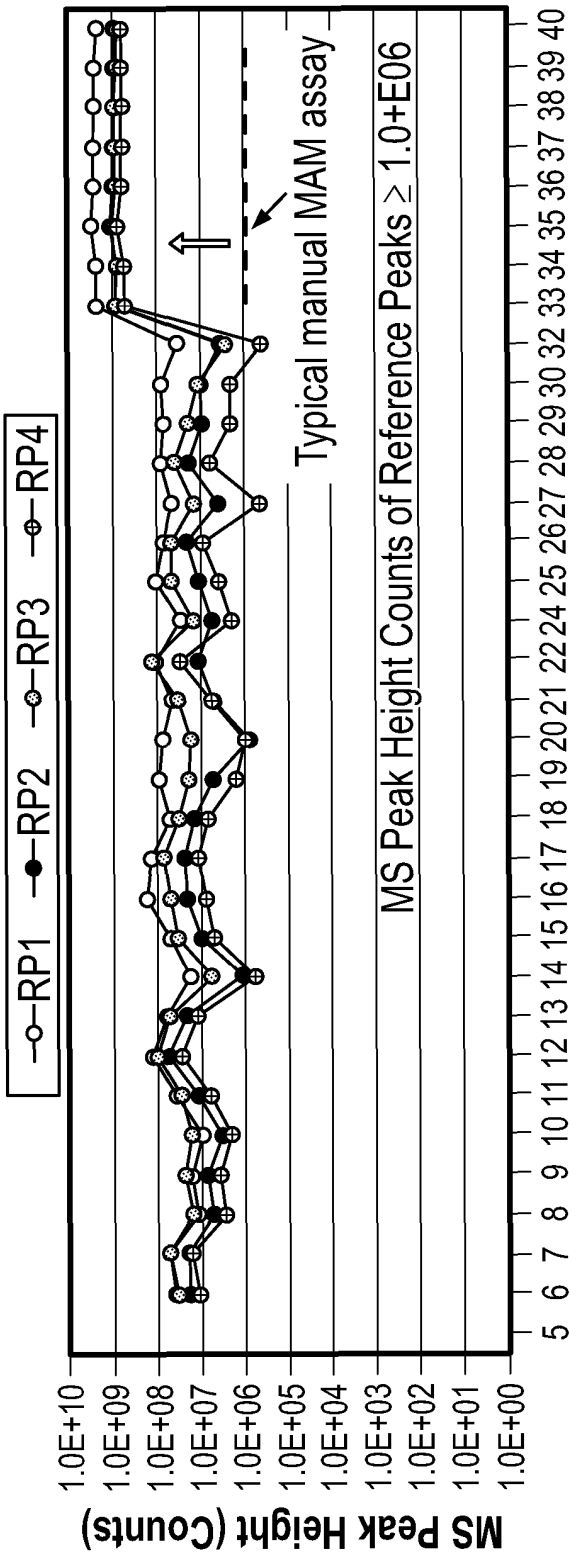
30 32 33 34 35 36 37 38 39 40

FIG. 3A


RP1
RP2
RP3
RP4
Typical manual MAM assay
MS Peak Height Counts of Reference Peaks ≥ 1.0+E06
MS Peak Height (Counts)
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1.0E+09
1.0E+08
1.0E+07
1.0E+06
1.0E+05
1.0E+04
1.0E+03
1.0E+02
1.0E+01
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Days of Production

FIG. 3B

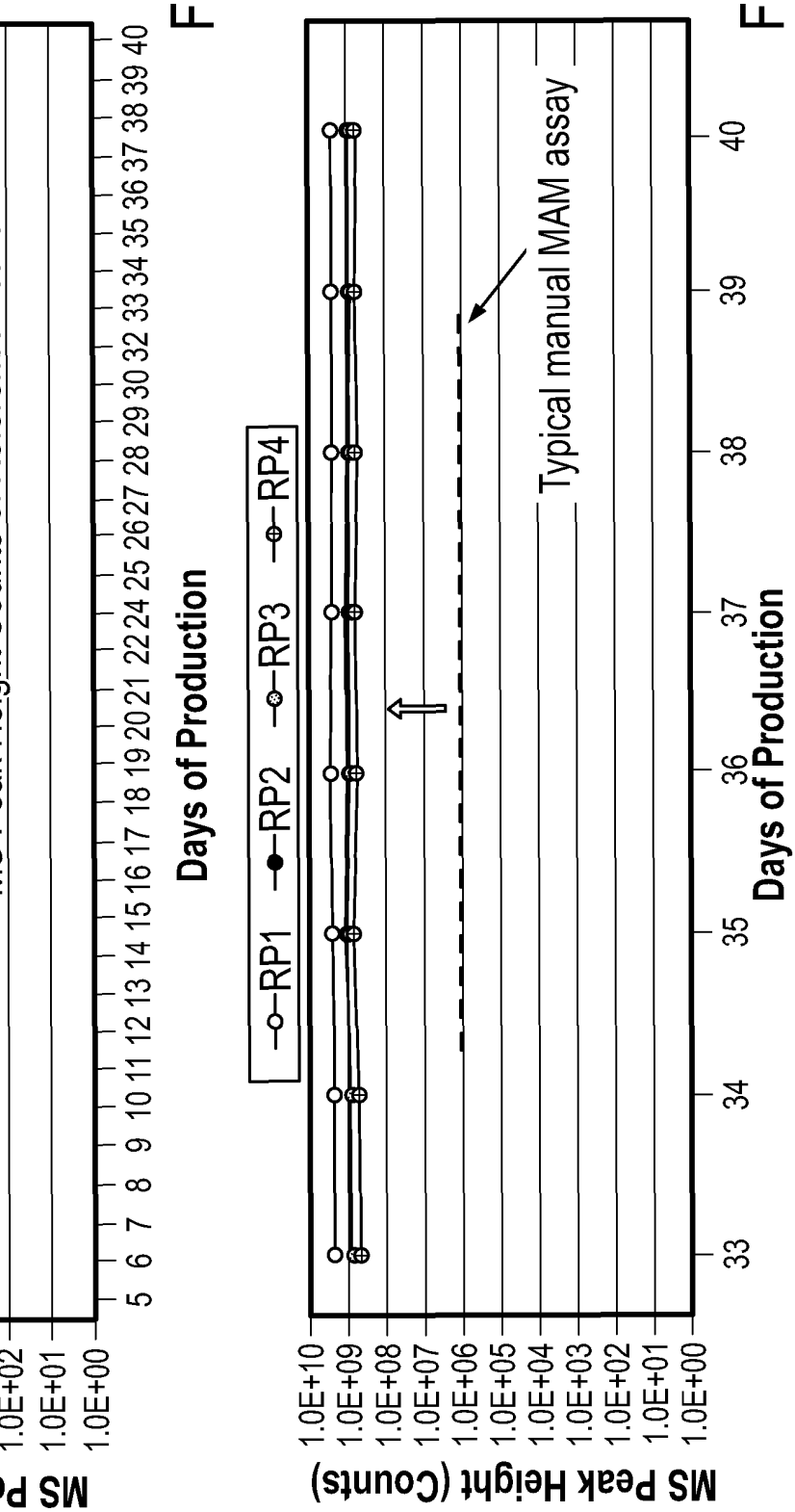
RP1
RP2
RP3
RP4
Typical manual MAM assay
MS Peak Height (Counts)
1.0E+10
1.0E+09
1.0E+08
1.0E+07
1.0E+06
1.0E+05
1.0E+04
1.0E+03
1.0E+02
1.0E+01
1.0E+00
33 34 35 36 37 38 39 40
Days of Production

FIG. 3C

REFERENCES CITED IN THE DESCRIPTION

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